HHS Public Access

Author manuscript

J Diet Suppl. Author manuscript; available in PMC 2024 January 01.

Published in final edited form as:

J Diet Suppl. 2023 ; 20(6): 850–869. doi:10.1080/19390211.2022.2128501.

Hepatotoxicity with High-Dose Green Tea Extract: Effect of *Catechol-O-Methyltransferase and Uridine 5′-Diphospho-glucuronosyltransferase 1A4* Genotypes

Laura Acosta, DCN, RDN, LDN,
Department of Clinical and Preventive Nutrition Sciences, Rutgers University School of Health Professions

Laura Byham-Gray, PhD, RDN, FNKF [Professor and Research Vice Chair],
Department of Clinical and Preventive Nutrition Sciences, Rutgers University School of Health Professions

Mindy Kurzer, PhD [Professor],
Department of Food Science and Nutrition, University of Minnesota

Hamed Samavat, PhD, RD [Assistant Professor]
Department of Clinical and Preventive Nutrition Sciences, Rutgers University School of Health Professions

Abstract

The predominant catechin in green tea, epigallocatechin gallate (EGCG), may be hepatotoxic in high doses. Our objective was to investigate the influence of *catechol-O-methyltransferase* (*COMT*) and *uridine 5′-diphospho-glucuronosyltransferase 1A4* (*UGT1A4*) genotypes on changes in liver injury biomarkers in response to long-term, high-dose green tea extract (GTE) supplementation among postmenopausal women. A secondary analysis was conducted using data from the Minnesota Green Tea Trial (N=1,075), in which participants were randomized to consume high-dose GTE (843 mg/day EGCG) or placebo capsules for 12 months. Analysis of covariance adjusting for potential confounders was performed to examine changes in aspartate aminotransferase (AST), alanine aminotransferase (ALT), AST: ALT ratio, and alkaline phosphatase from baseline to months 3, 6, 9, and 12 across *COMT* and *UGT1A4* genotypes. Mean age and BMI within the GTE group (n=400) were 59.8 years and 25.1 kg/m^2, respectively, and 98% of subjects were white. From baseline to month 3, mean AST: ALT ratio change was +1.0% in the *COMT* (rs4680) *A/G* genotype versus −4.8% in the *A/A* genotype (P=0.03). From baseline to months 6 and 9, respectively, mean ALT change was +78.1% and +82.1% in the *UGT1A4* (rs6755571) *A/C* genotype versus +28.0% and +30.1% in the *C/C* genotype (P<0.001

Corresponding Author: **Laura Acosta, DCN, RDN, LDN,** Department of Clinical and Preventive Nutrition Sciences, Rutgers University School of Health Professions, ljacosta@ufl.edu.

Declaration of Interest:
The authors disclose no conflicts of interest.

Consent:
As a condition of participation in the Minnesota Green Tea Trial (the parent study for this secondary analysis), subjects signed an informed consent prior to any data collection. Therefore, all data that were used for this secondary analysis were obtained under consent from the subjects in the parent study.

and *P*=0.004, respectively). The *UGT1A4* (rs6755571) *A/C* genotype may be an important risk factor for clinically-relevant serum transaminase elevations with 6–9 months of high-dose GTE supplementation among postmenopausal women. Understanding the genetic underpinnings of GTE-related hepatotoxicity may allow for a genetically-informed paradigm for therapeutic use of GTE.

Introduction:

Green tea, produced from the *Camellia sinensis* plant, has been used for medicinal purposes in east Asia for thousands of years,[1] and in the past several decades has shown promise for its potential to mitigate risk for chronic health conditions such as cancer,[2–5] cardiovascular disease,[6] obesity,[7,8] and insulin resistance.[9] The primary driver of green tea's health-promoting effects is thought to be the potent antioxidant properties of its predominant catechin, epigallocatechin gallate (EGCG).[10] There is concern, however, that EGCG may be hepatotoxic in high doses.[11]

Hepatotoxicity, or chemically-induced liver injury (from pharmaceuticals, supplements, medicinal herbs, etc.), affects approximately 50 million people globally and increases the risk for long-term liver complications such as hepatocellular carcinoma, cirrhosis, and hepatitis C.[12] The Drug-Induced Liver Injury Network (DILIN), involving eight United States medical centers, is an ongoing registry for hepatotoxic events resulting from pharmaceutical agents as well as herbal and dietary supplements.[31] Causality assessment revealed that out of 1,414 patients in the DILIN with confirmed liver injury between 2004 and 2018, 70 had suspected exposure to green tea extract (GTE), and 40 cases were directly attributable to GTE consumption.[13] Due to the potential for GTE-related liver damage, food safety agencies such as the European Food Safety Authority Panel on Food Additives and Nutrient Sources Added to Food (EFSA ANS) have recommended limiting GTE consumption to doses providing <800 mg/day of EGCG.[11]

The degree to which high-dose EGCG will cause hepatotoxicity in a given individual, and whether it will at all, is challenging to predict. Yu et al.[14] found that liver enzyme spikes affected only about 5% of women taking a high-dose GTE supplement for 12 months, and there were no significant demographic or lifestyle differences between subjects who experienced enzyme elevations and those who did not. In a single group futility study by Lovera et al.[15] only one subject (out of 10) taking a high-dose green tea catechin mixture experienced mild liver enzyme elevations. However, when the authors conducted a follow up randomized controlled trial (RCT) using the same product, 83% of subjects taking the catechins developed severe liver enzyme derangements, and the trial was terminated early.[15]

Genetic variation may explain some of the inter-individual variability in hepatic response to high-dose GTE. The enzyme catechol-*O*-methyltransferase (COMT) metabolizes EGCG through *O*-methylation.[16] A single nucleotide polymorphism (SNP rs4680) in the *COMT* gene, a guanine (*G*) to adenine (*A*) substitution at codon 158, results in the replacement of valine with methionine. The *A* allele presents with different frequency across races and ethnicities, and has a prevalence of approximately 63% among Mexican populations, 54% among white populations, 34% among African populations, and 29% among Asian

populations.[17] This variant allele reduces the thermal stability of the enzyme and is thought to reduce its activity by nearly 40 percent.[16,18] We hypothesized that the reduced metabolic efficiency of COMT resulting from this polymorphism may prolong hepatic exposure to EGCG and increase the risk for hepatotoxicity with high-dose GTE (Figure 1). While O-methylation by COMT is the predominant metabolic pathway for EGCG degradation, smaller fractions of EGCG are metabolized via sulfation by sulfotransferase 2A1 (SULT2A1) and glucuronidation by uridine 5′-diphospho-glucuronosyltransferase 1A4 (UGT1A4).[19–21] Thus polymorphisms in the *SULT2A1* and *UGT1A4* genes could theoretically affect efficiency of EGCG metabolism as well, and have implications for hepatotoxicity risk. However, to date, genetic risk factors for hepatotoxicity with high-dose GTE have been largely unexplored.

Elevated blood concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AKP), in conjunction with the ratio of AST: ALT, are conventionally used to identify liver injury and provide insight on its etiology.[22] Collectively, these biomarkers are often referred to as liver function tests (LFTs). This term misrepresents their role however, as they are indicators of liver injury, not measures of liver "function".[23] While references ranges vary slightly among laboratories, serum concentrations of approximately 10–35 U/L for AST and 6–60 U/L for ALT are considered normal.[14,24] Elevations in AST and ALT, transaminases that transfer amino groups to α-ketoglutarate, are characteristic of hepatocellular injury.[25–28] When hepatocytes are damaged, their cell membranes become more permeable and leak AST and ALT into circulation, resulting in elevated serum transaminase concentrations.[23] Both enzymes are abundant in the liver, but because AST is found in many other organs as well, including the brain, pancreas, heart, lungs, and kidneys, ALT is considered to be more liver-specific.[29] A concentration of approximately 33–130 U/L is considered normal for AKP.[14,24] Elevated AKP is characteristic of cholestatic injury, or impaired bile secretion, which can lead to excessive hepatic accumulation of bile acids.[30] While GTE-related hepatotoxicity is most often hepatocellular in nature, cholestatic injury has been documented in up to one-third of cases,[30] suggesting that the mechanisms and manifestations of GTE-induced liver damage may be multilayered.

Women are more likely than men to experience hepatotoxicity, likely due in part to sex-mediated differences in the bioavailability, metabolism, and excretion of drugs and other bioactive compounds.[31,32] Postmenopausal women may be even more vulnerable to liver pathology as age-related changes in liver structure and function can significantly impact clearance capacity,[33] and estrogen loss can result in derangements of mitochondrial integrity, antioxidant function, and immune defenses.[34]

To our knowledge, no previous studies have examined the effects of GTE on liver injury biomarkers exclusively in postmenopausal women and through a nutritional genetics lens. Therefore, the objective of this study was to fill an important gap in the literature by investigating the influence of *COMT* and *UGT1A4* genotypes on changes in AST, ALT, AST: ALT ratio, and AKP in response to long-term, high-dose GTE supplementation among healthy, postmenopausal women.

Methods:

We conducted a secondary analysis using data from the Minnesota Green Tea Trial (MGTT), a large (N=1,075) double-blinded, placebo-controlled trial in which participants were randomized to consume either high-dose GTE or placebo capsules daily for 12 months. The study design and methods for the MGTT are described in detail elsewhere.[35] Briefly, postmenopausal women aged 50–70 years with high mammographic density were recruited and screened for eligibility. Potential subjects were excluded if they: were unwilling to provide written informed consent; consumed green tea regularly (more than one cup per week); had preexisting liver complications (Hepatitis B, Hepatitis C, or elevated liver enzymes >1.5 times the upper limit of normal); were currently using or had recently used hormone therapy; had a history of cancer (any kind) in the past five years; ever had breast cancer, ovarian cancer, or proliferative breast disease; had breast implants; were currently taking methotrexate or etanercept; were enrolled in a weight management program; had experienced a weight change of over ten pounds in the past one year; or currently smoked or used tobacco products. Qualified subjects underwent genotyping for *COMT* (SNP rs4680) and *UGT1A4* (SNPs rs10929293, rs10929301, rs11673726, rs17862875, rs2011404, and rs6755571) at baseline in the parent study. DNA was extracted from the buffy coat using a Qiagen DNeasy Blood and Tissue Kit manufactured by Qiagen, Inc. (Gaithersburg, MD), and a TaqMan PCR Core Reagent Kit manufactured by Applied Biosystems (Foster City, CA) was used for genotyping. SNP analysis was performed by the University of Minnesota Genomics Center on Sequenom iPlex platform (Sequenom, San Diego, CA).[35]

A total of 1,075 subjects who met eligibility criteria were randomized to consume either GTE capsules (843 ± 44 mg/day EGCG) or maltodextrin-cellulose placebo capsules for 12 months. Each day, two capsules were taken twice with breakfast and dinner, for a total of four capsules per day. Although the originally intended dose of EGCG was 800 mg/day, eight different batches of the GTE product were used during the trial and upon high performance liquid chromatography analysis, the mean EGCG dose used was 843 mg/day.[35] This discrepancy was not determined to be clinically significant, and it was not considered problematic by National Institutes of Health (NIH)/National Cancer Institute reviewers or the study's Data Safety Monitoring Board.

Serum concentrations of AST, ALT, and AKP were measured at baseline, and monitored with hepatic panels every three months (months 3, 6, 9, and 12).[35] Any ALT value >90 U/L (corresponding to >1.5 times the upper limit of normal) was treated as an adverse event, and subjects were required to immediately stop taking study capsules. If the elevated ALT value was 1.5–5.0 times the upper limit of normal (90–300 U/L), a follow up hepatic panel was conducted every two weeks following the initial detection of elevated ALT, and the subject was allowed to restart taking the study capsules if/when their ALT returned to <90 U/L. If ALT was elevated to >300 U/L (>5 times the upper limit of normal), the subject was withdrawn from the study and GTE consumption was permanently discontinued.[14]

The present study was a secondary analysis of data from the MGTT, and all participants in the parent study provided written informed consent. Data were included from subjects who met criteria for the parent study and for whom genotype data and liver enzyme data

were available. Due to potential effects of body weight and alcohol intake on liver enzyme concentrations, data were excluded from subjects with BMI <18.5 or >40 kg/m^2, and from those whose baseline alcohol consumption exceeded 14 g ethanol per day. The following variables were extracted for use in the secondary analysis. Continuous variables included age, body mass index (BMI), alcohol intake (at baseline and month 12), dietary intake (energy, carbohydrate, protein, total fat, and saturated fat at baseline and month 12), and liver enzymes (AST, ALT, and AKP at baseline and months 3, 6, 9, and 12). Categorical variables included race, ethnicity, smoking status (former or never), number and type(s) of medications used, type(s) of herbal supplements consumed more than once per week, *COMT* genotype, and *UGT1A4* genotype. *COMT* genotype (SNP rs4680) was treated as a categorical nominal variable with three groups; *G/G* (wild-type), *A/G* (heterozygous), and *A/A* (homozygous variant). Six SNPs for *UGT1A4* genotype (SNPs rs10929293, rs10929301, rs11673726, rs17862875, rs2011404, and rs6755571) were each treated as categorical nominal variables with three groups; wild-type, heterozygous, and homozygous variant (allelic variations are shown in Figure 2).

Medication data were reviewed to identify subjects who reported taking medications known to affect the liver, including aspirin, acetaminophen, non-aspirin NSAIDs, antibiotics, statins, antiarrhythmic medications, blood pressure medication, and anti-depressants/anti-psychotics. Additionally, supplement use data were reviewed to identify subjects who reported taking herbal supplements (more than once per week) known to affect the liver, including aloe vera, cascara sagrada, feverfew, and kava. AST: ALT ratio was calculated by dividing AST by ALT at each time point. Relative changes in AST, ALT, AST: ALT ratio, and AKP from baseline to months 3, 6, 9, and 12 were calculated by subtracting the baseline value from the final value for each time interval, then dividing the difference by the baseline value, and multiplying by 100 to get percent (relative) change.

Statistical Analyses:

For continuous variables, normality was assessed with histograms and box-and-whisker plots. Continuous variables were reported using mean ± standard deviation or median (Interquartile range) depending on data distribution, and categorical variables were presented using frequency (%). To identify potential confounders, bivariate analyses were performed using correlation test for continuous variables, and independent samples t-test or analysis of variance [ANOVA] for categorical variables for the baseline liver enzyme concentrations and study population characteristics. To examine the differences in AST, ALT, AST: ALT ratio, and AKP across *COMT* and *UGT1A4* genotypes at baseline for the entire sample, analysis of covariance (ANCOVA) was performed adjusting for baseline age, BMI, ethnicity, smoking status, and dietary intake (energy, carbohydrate, fat, and alcohol). The same ANCOVA model was used to evaluate changes in AST, ALT, AST: ALT ratio, and AKP from baseline to months 3, 6, 9, and 12 across *COMT* and *UGT1A4* genotypes in response to the high-dose GTE supplement. The ANCOVA analyses were re-run with adjustment for medication and herbal supplement use to determine whether inclusion of these variables would significantly change the results. Additionally, to examine the overall effect of time and differences in AST, ALT, AST: ALT ratio, and AKP across five time points, we performed separate repeated measures ANOVA tests adjusting for baseline age,

BMI, ethnicity, smoking status, and dietary intake (energy, carbohydrate, fat, and alcohol) for each *COMT* (rs4680) genotype and each of the six SNPs of the *UGT1A4* genotype. A general linear model was also used to evaluate interactions between time and genotype for each hepatic biomarker. A *post hoc* power analysis was performed on the primary aim using ANCOVA test (fixed effects, main effects, and interactions) in the G*Power software (version 3.1.9.7)[36] assuming an alpha level of 0.05 and an existing sample size of 799, which showed this study had more than 80% statistical power to detect a significant difference in baseline liver enzyme concentrations across *COMT* genotypes. All statistical analyses were performed using IBM® SPSS (Version 28.0, IBM Inc., Armonk, NY). An *a priori* alpha level of $P \leq 0.05$ was used to indicate statistical significance.

Results:

Of the 1,075 subjects in the MGTT, 13.1% (n=141) did not have liver enzyme data available at all time points and were excluded from the secondary analysis. An additional 12.4% (n=133) were excluded for alcohol consumption in excess of 14 g per day, and 0.2% (n=2) were excluded for BMI <18.5 or >40 kg/m^2. The final sample for the secondary analysis was N=799 (Figure 3), with n=400 in the GTE treatment group.

The results presented henceforth are for the GTE group, unless otherwise noted. Mean age and BMI were 59.8 years and 25.1 kg/m^2, respectively, and 98% (n=391) of subjects were white and non-Hispanic. At baseline, 92.3% (n=369) of subjects reported taking medications known to affect liver function, whereas only 0.5% (n=2) reported taking herbal supplements (aloe vera, cascara sagrada, feverfew, or kava). Median baseline alcohol intake was 2.4 g ethanol per day, and 69% of subjects had never smoked. Mean baseline energy intake was 1419 kcal/day, composed of approximately 51% carbohydrate, 16% protein, and 33% fat. There were no significant differences in baseline demographic characteristics across *COMT* genotypes (Table 1) or *UGT1A4* genotypes (data not shown). There were significant weak positive correlations between baseline AST and age; baseline AKP and age; baseline AKP and BMI; and baseline AST: ALT ratio and carbohydrate intake (Supplemental Table S1). There were significant weak negative correlations between baseline AST and BMI; baseline AKP and alcohol intake; and baseline AST: ALT ratio and BMI, energy intake, and total fat intake (Supplemental Table S1). Similar correlations were found in the full sample. Mean AST, ALT, and AKP concentrations were within normal ranges at baseline, while mean AST: ALT ratio was slightly elevated at 1.2 (normal <1.0). There were no significant differences in hepatic biomarker concentrations by demographic characteristics, medication and supplement use, or smoking status at baseline, either in the GTE group (Supplemental Table S2) or in the full sample (data not shown).

Adjustment for medication and herbal supplement use at baseline did not change the results for any of the ANCOVA tests. There were no significant differences in baseline AST, ALT, AST: ALT ratio, or AKP across *COMT* genotypes (Table 2) or across any of the *UGT1A4* genotypes (data not shown) in the entire sample. There was a significant difference in change in AST: ALT ratio from baseline to month 3 between the *A/G* and *A/A COMT* (rs4680) genotypes in the GTE group (Table 3). In this time period, mean AST: ALT ratio increased by 1.0% in the *A/G* genotype while it decreased by 4.8% in the *A/A*

genotype (P=0.03). There were no significant differences in any other hepatic biomarker changes for any other time intervals across *COMT* genotypes in the GTE group (Table 3). The effect of time was significant for AST, ALT, and AST: ALT ratio within all *COMT* (rs4680) genotypes in the GTE group, although the specific time points at which enzyme concentrations were significantly different varied among genotypes (Table 4). There was notable variability in both AST and ALT at months 3 and 12 in the *A/G* genotype, and at month 3 in the *A/A* genotype. Upon exploratory analysis using the same repeated measures ANOVA tests in the placebo group for comparison, there was very little variability or change over time for any *COMT* (rs4680) genotype in the placebo group (Supplemental Figures S1 and S2). There were no significant interactions between time and *COMT* genotype for any of the hepatic biomarkers.

The effect of *UGT1A4* polymorphisms (rs10929293, rs10929301, rs11673726, rs17862875, rs2011404, and rs6755571) on hepatic markers were also investigated within the GTE group. For the *UGT1A4* (rs11673726) SNP, there was a significant difference for change in AKP from baseline to month 6 between the *T/G* and *T/T* genotypes (Supplemental Table S3). In this time period, mean AKP decreased by 1.8% in the *T/G* genotype while it increased by 3.6% in the *T/T* genotype (P=0.01). Changes in hepatic biomarkers from baseline to months 3, 6, 9, and 12 by *UGT1A4* (rs6755571) genotype are shown in Table 5. There were significant differences for changes in AST and ALT from baseline to months 6 and 9 between the *C/C* and *A/C* genotypes. From baseline to month 6, mean AST increased by 36.3% in the *A/C* genotype compared to 15.9% in the *C/C* genotype (P=0.02), and ALT increased by 78.1% in the *A/C* genotype compared to 28.0% in the *C/C* genotype (P<0.001). From baseline to month 9, mean AST increased by 41.5% in the *A/C* genotype compared to 15.8% in the *C/C* genotype (P=0.01), and ALT increased by 82.1% in the *A/C* genotype compared to 30.1% in the *C/C* genotype (P=0.004). For the remaining four *UGT1A4* SNPs (rs10929293, rs10929301, rs17862875, and rs2011404), there were no significant differences across genotypes in any hepatic biomarkers for any time interval.

The effect of time was significant for AST in all *UGT1A4* genotypes except rs17862875 *A/A*, rs11673726 *T/T*, and rs2011404 *T/T*; and for ALT in all *UGT1A4* genotypes except rs17862875 *A/A* and rs11673726 *T/T* (data not shown). There were no significant interactions between time and any of the *UGT1A4* genotypes for any of the hepatic biomarkers.

Discussion:

This secondary analysis examined the effects of *COMT* and *UGT1A4* genotypes on hepatic response to high-dose GTE supplementation among healthy postmenopausal women. Our findings demonstrate that individuals with the *UGT1A4* (rs6755571) *A/C* genotype may experience significant elevations in transaminase concentrations relative to the *UGT1A4* (rs6755571) *C/C* genotype from baseline to months 6 and 9; and that individuals with the *COMT* (rs4680) *A/A* genotype may experience significant reductions in the AST: ALT ratio relative to the *COMT* (rs4680) *A/G* genotype from baseline to month 3.

GTE-induced liver damage, while relatively rare, is an important clinical problem when it does occur. Liver biopsies from patients in the DILIN with hepatotoxicity attributed to GTE revealed inflammation, cholestasis, steatosis, and necrosis.[13] The degree to which high-dose EGCG will cause hepatotoxicity in a given individual, and whether it will at all, is challenging to predict. Clear demographic and clinical risk factors remain elusive,[14,15] and prior to the current study, little was known about genetic factors driving the inter-individual variability in GTE-related liver pathology. Only one previous study to date had explored *COMT* genotype in relationship to GTE-induced hepatotoxicity, and it was limited in scope as ALT was the only biomarker evaluated.[37] No previous studies had explored *UGT1A4* genotype in this context, and there was no literature describing whether or how *UGT1A4* polymorphisms may affect catechin metabolism.

Our analysis revealed few differences overall in hepatic response to GTE across genotypes. There were, however, a few notable exceptions, suggesting that changes in hepatic biomarkers over time among postmenopausal women taking high-dose GTE supplements may depend in part on genetic factors.

Serum Transaminases

There were significant differences across *UGT1A4* (rs6755571) genotypes in AST and ALT in response to GTE supplementation. At months 6 and 9, the relative increases from baseline in AST and ALT were approximately 2.5 times greater in the heterozygous *A/C* genotype compared to the wild-type *C/C* genotype. Although mean AST and ALT values remained within normal limits at all time points, it is important to consider that the defined normal values for many laboratory tests, including serum transaminases, are based on the values found within approximately 95 percent of the population[23] and not necessarily the thresholds that are ideal or “healthy”. Mortality data suggest that even within “normal” laboratory ranges, there is a positive correlation between circulating ALT concentrations ≥20 U/L and liver-related mortality.[38] It has been proposed that an upper limit of 19 U/L in women (and 30 U/L in men) may be a more appropriate threshold to reflect “healthy” ALT concentrations, compared to the commonly used upper limit of “normal” (~60 U/L).[39] Against this backdrop, our findings suggest that even when AST and ALT stay within normal limits by laboratory standards, those individuals with the heterozygous *A/C* genotype for *UGT1A4* (rs6755571) may be particularly vulnerable to clinically-relevant serum transaminase elevations with 6–9 months of high-dose GTE supplementation. In future research, it may be interesting to explore differences in hepatic markers by *UGT1A4* genotypes among individuals who experience liver enzyme elevations above upper normal limits in response to GTE.

AST: ALT Ratio

The AST: ALT ratio (or De Ritis ratio), originally proposed as a diagnostic test for viral hepatitis,[40] is now utilized for etiological insight and differential diagnosis in a variety of liver pathologies.[23] The AST: ALT ratio is normally <1.0, meaning that in the absence of liver pathology AST concentration is typically lower than ALT concentration.[41] In the present study, mean AST: ALT ratio at baseline was 1.2, suggesting that AST was elevated relative to ALT. While an AST: ALT ratio of 1.2 is considered slightly high for the general

population, we were not surprised by this finding given that our data came from a sample of postmenopausal women. Both AST and ALT tend to increase early in the menopause transition. ALT later decreases while AST remains elevated, resulting in an elevated AST: ALT ratio, and this is considered common in post-menopause.[42]

There was a significant difference in the relative change in AST: ALT ratio from baseline to month 3 between the *COMT* (rs4680) *A/G* and *A/A* genotypes. The ratio increased by 1.0% in the heterozygous *A/G* genotype while it decreased by 4.8% in the homozygous variant *A/A* genotype in the same three-month time frame. Because ALT is more liver-specific than AST, and a decrease in AST: ALT ratio would imply that ALT increased relative to AST, we might deduce from this finding that the GTE supplement had a greater hepatic effect in those with the *A/A* genotype compared to the *A/G* genotype in the first three months of supplementation. This difference was no longer significant by month 6 and remained non-significant thereafter.

In perspective however, these findings, while intriguing, are not likely to be clinically relevant. A 1.0% increase in a baseline AST: ALT ratio of 1.2 would yield a ratio of 1.212, which is clinically indistinguishable from 1.2. Perhaps the more important implication of these findings is that they challenge our understanding of the phenotypic distinctions among the *COMT* (rs4680) genotypes. Because presence of the variant *A* allele is thought to reduce COMT enzyme activity, the three genotypes have historically been described as "high-activity" (*G/G*, wild-type), "intermediate-activity" (*A/G*), and "low-activity" (*A/A*).[43] In previous studies, including in our own previous work, the *A/A* and *A/G* genotypes have been combined for analytical purposes and collectively characterized as "reduced-activity".[35,44] Thus we expected that hepatic biomarker changes would be similar in the *A/G* and *A/A* genotypes, and that if significant differences existed they would likely present between the wild-type *G/G* genotype and one or both of the variant genotypes. Instead, the only significant difference we found across the *COMT* (rs4680) genotypes was between the two supposed "reduced-activity" variants, and neither was significantly different from the "high-activity" genotype. This finding challenges the premise that the *A/G* and *A/A* genotypes can be considered similar, and suggests that the effect of *COMT* genotype on AST: ALT ratio with high-dose GTE may be more complex than can be captured with simple characterizations of enzyme activity levels. It is possible that other genetic and non-genetic factors not controlled for in this study may have influenced these findings as well. For instance, in this study we examined genotype SNPs separately, but there may be synergistic interactions among SNPs that together either lessen or exaggerate liver enzyme changes with GTE. Additionally, the activity of catechin metabolizing enzymes that were not analyzed in this study, such as SULT2A1, could have influenced hepatic outcomes as well. Exposure to environmental toxins, and behavioral factors such as physical activity, which were not controlled for in this study, may also influence the effect of GTE on the liver. In future research, it may be insightful to explore additional genetic and non-genetic variables, and potential interactions among SNPs.

Alkaline Phosphatase

Elevated AKP (>2 times the upper limit of normal) is indicative of cholestatic injury[30] which sometimes occurs with high-dose GTE, albeit less commonly than hepatocellular injury. We found statistically significant differences across *UGT1A4* (rs11673726) genotypes, specifically between the *T/G* (heterozygous) and *T/T* (homozygous) variants, for changes in AKP from baseline to month 6. However, mean AKP values never rose to levels consistent with cholestasis, and the AKP changes themselves, while statistically significant, were relatively small (−1.8% to 3.6%) and unlikely to have clinical relevance. Thus, our findings do not implicate *UGT1A4* (rs11673726) genotype as a risk factor for cholestatic injury with GTE.

Effect of Time

Time had a significant effect on hepatic biomarkers within all genotypes, which was not unexpected given the known hepatic implications of high-dose GTE supplementation. What was surprising was the non-linear patterns of biomarker fluctuation across time points. Rather than rising steadily over time, transaminase concentrations stayed similar to baseline until, in most cases, approximately month 6. They would peak around months 6–9, and then remain stable or even fall slightly by month 12. Because the inter-individual variability in transaminase concentrations at each time point was very limited within the placebo group, we are confident that the high variability we observed within the *COMT* (rs4680) *A/C* genotype at months 3 and 12 and the *A/A* genotype at month 3 was attributable to the GTE supplement. We did not find any significant interactions between time and genotype, suggesting that regardless of genotype, the acute hepatic effects of high-dose GTE are largely seen in the first 6–9 months of supplementation, after which a level of tolerance may develop. This phenomenon should be further explored in future research.

Our findings of high inter-individual variability align with similar observations in contemporary literature on bioactive compounds. A review by Milenkovic et al.[45] concluded that cardiometabolic responses to polyphenols, catechins, caffeine and plant sterols are highly variable, and mediated in part by genetic polymorphisms, in addition to other factors such as sex, age, disease, gut microbiome, and metabolic factors. Other authors echo the observation that individual responses to bioactive compounds vary considerably, and that a deeper understanding of the determinants of variability is necessary to develop a robust, evidence-based approach to personalized nutrition.[46–47]

Strengths and Limitations

To our knowledge, this study is only the second to investigate the effect of *COMT* genotype on hepatic response to GTE. Dostal et al.[37] evaluated adverse effects observed in the MGTT, including acute ALT elevation, and stratified results by *COMT* genotype. No significant differences in ALT elevation were found across genotypes, but the study was limited in scope as ALT was the only hepatic biomarker analyzed and *COMT* (rs4680) was the only SNP considered. No previous studies, to our knowledge, have explored the implications of *UGT1A4* genotype for GTE-related hepatotoxicity risk. Besides the MGTT, only one other RCT has examined the effects of green tea in postmenopausal women exclusively and included data on liver enzyme changes. Shen et al.[48] concluded that green tea polyphenols

at a dose of 500 mg/day for 24 weeks appeared to be safe and did not significantly alter mean liver enzyme concentrations. However, genetic variables or stratification of results by genotype were not considered in this trial. Thus, our approach was novel, as we explored hepatic response to GTE in postmenopausal women through a nutritional genetics lens.

This study did have some limitations. The MGTT was not originally designed to evaluate hepatic biomarkers as primary outcomes. Measurements could not be retaken, and no additional data could be collected, as we had no access to subjects. Furthermore, medication use data were only collected at baseline and we were not able to ascertain whether medication use remained consistent throughout the 12-month trial. An additional limitation is the fact that MGTT subjects who experienced severely elevated ALT were withdrawn from the trial. There is a possibility that this could have skewed our results, as we had no way of knowing what concentrations their ALT (and other liver enzymes) may have reached had they continued taking the GTE capsules. Any effect this may have had on our results was likely minimal however, as severe ALT elevation necessitating withdrawal from the MGTT occurred in only eight participants.[14] It would be interesting in future research to examine what effect *COMT* and/or *UGT1A4* genotypes might have on the time it takes for transaminase concentrations to return to baseline levels after discontinuing high-dose GTE. Finally, the generalizability of this analysis may be limited. The sample in the MGTT was relatively homogenous, and composed of predominantly Caucasian and non-Hispanic postmenopausal women. Our results therefore may only be generalizable to a population of healthy, Caucasian postmenopausal women living in a metropolitan area in the north-central United States. Because hepatic clearance capacity decreases with age, we speculate that we may see less dramatic transaminase elevations in response to GTE if this same study were conducted in younger women. This may be an interesting hypothesis to explore in future research.

Implications

With global healthcare costs at a staggering all-time high (the equivalent of $7.8 trillion US dollars as of 2017, according to the World Health Organization[49]), there is a clear economic rationale for development of cost-effective treatments and preventive measures for major morbidities such as cardiovascular disease, cancer, diabetes, and obesity. Green tea has shown promise as an adjunctive therapy for many of these conditions, and in some cases, relatively high doses are needed to achieve a clinically significant effect. Current guidelines recommend limiting GTE to doses providing <800 mg/day of EGCG due to concerns for hepatotoxicity,[11] even though the proportion of individuals that experience hepatotoxic effects with high-dose GTE is relatively low. Thus we purport that green tea is currently underutilized as a clinical intervention and its therapeutic potential has yet to be fully realized. Genotyping is efficient and inexpensive using modern techniques. For instance, a novel quantitative polymerase chain reaction (qPCR) assay developed by Ojeda et al.[50] for *COMT* (rs4680) genotyping allows for analysis of nearly 100 samples in two hours for under 30 dollars. Thus genotyping for catechin-metabolizing enzymes may be both feasible and informative for individuals considering high-dose GTE supplementation. In particular, it may be prudent to consider genotyping for *UGT1A4* (rs6755571) when GTE supplementation is anticipated to last at least 6–9 months, at a dose of 800+ mg/day EGCG.

In a rapidly-evolving era of precision medicine, understanding the genetic underpinnings of GTE-related liver enzyme changes may allow us to embrace the therapeutic potential of GTE with confidence, and use it judiciously to maximize benefit without causing harm.

Conclusion:

In conclusion, the results of our analysis suggest that the *UGT1A4* (rs6755571) *A/C* genotype may be an important risk factor for clinically-relevant serum transaminase elevations with 6–9 months of high-dose GTE supplementation among postmenopausal women. Furthermore, there may be phenotypic differences between the *COMT* (rs4680) *A/G* and *A/A* variants that affect the AST: ALT ratio in the first three months of GTE supplementation. While the *A/G* and *A/A COMT* genotypes have been combined for analytical purposes in previous research, our findings challenge the assumption that *A/G* and *A/A* can be considered similar, and it may be best practice for future studies to separate the three genotypes as distinct categories. *UGT1A4* (rs11673726) genotype may slightly affect how AKP changes in the first six months of high-dose GTE consumption. However, differences across the allelic variations are likely clinically irrelevant and do not implicate *UGT1A4* (rs11673726) genotype as a meaningful risk factor for GTE-induced cholestatic injury.

Although green tea has shown promise as an adjunctive therapy for numerous chronic health conditions, fears of hepatotoxicity have likely stifled broad clinical adoption. Up to this point, there has been little investigation of genetic factors that influence the risk for liver injury following consumption of GTE supplements, and out of caution there have been blanket recommendations to limit EGCG consumption to <800 mg/day. However, the literature shows that only a small proportion of individuals experience liver injury with high-dose EGCG, and many tolerate it without issue.[14–15] While more research is needed to fully and precisely elucidate the factors that put certain individuals at higher risk for liver injury with high-dose GTE, we believe our analysis provides valuable insights to prompt discussion around genetically-informed approaches to the therapeutic use of GTE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding:

This work was supported by the National Institutes of Health (NIH) / National Cancer Institute under Grant R01 CA127236

Data Availability Statement:

Data will be made available on reasonable request.

REFERENCES

1. Yang CS, Chen G, Wu Q. Recent scientific studies of a traditional chinese medicine, tea, on prevention of chronic diseases. J Tradit Complement Med. 2014;4(1):17–23. DOI: 10.4103/2225-4110.124326. [PubMed: 24872929]
2. Yuan J-M. Cancer prevention by green tea: evidence from epidemiologic studies. American J Clin Nutr. 2013;98(6):1676S–1681S. DOI: 10.3945/ajcn.113.058271.
3. Huang Y, Chen H, Zhou L, et al. Association between green tea intake and risk of gastric cancer: a systematic review and dose-response meta-analysis of observational studies. Public Health Nutr. 2017;20(17):3183–3192. DOI: 10.1017/S1368980017002208. [PubMed: 28980522]
4. Man GCW, Wang J, Song Y, et al. Therapeutic potential of a novel prodrug of green tea extract in induction of apoptosis via ERK/JNK and Akt signaling pathway in human endometrial cancer. BMC Cancer. 2020;20(1):964. DOI: 10.1186/s12885-020-07455-3. [PubMed: 33023525]
5. Guo Y, Zhi F, Chen P, et al. Green tea and the risk of prostate cancer: a systematic review and meta-analysis. Medicine. 2017;96(13):e6426. DOI: 10.1097/MD.0000000000006426. [PubMed: 28353571]
6. Nagao T, Hase T, Tokimitsu I. A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. Obesity. 2007;15(6):1473–83. DOI: 10.1038/oby.2007.176. [PubMed: 17557985]
7. Hill AM, Coates AM, Buckley JD, Ross R, Thielecke F, Howe PR. Can EGCG reduce abdominal fat in obese subjects? J Am Coll Nutr. 2007;26(4):396S–402S. DOI: 10.1080/07315724.2007.10719628. [PubMed: 17906193]
8. Lin Y, Shi D, Su B, et al. The effect of green tea supplementation on obesity: A systematic review and dose-response meta-analysis of randomized controlled trials. Phytother Res. 2020;34(10):2459–2470. DOI: 10.1002/ptr.6697. [PubMed: 32372444]
9. Fukino Y, Shimbo M, Aoki N, Okubo T, Iso H. Randomized controlled trial for an effect of green tea consumption on insulin resistance and inflammation markers. J Nutr Sci Vitaminol. 2005;51(5):335–42. DOI: 10.3177/jnsv.51.335. [PubMed: 16392704]
10. Singh BN, Shankar S, Srivastava RK. Green tea catechin, epigallocatechin-3-gallate (EGCG): Mechanisms, perspectives and clinical applications. Biochem Pharmacol. 2011;82(12):1807–1821. DOI: 10.1016/j.bcp.2011.07.093. [PubMed: 21827739]
11. Younes M, Aggett P, Aguilar F, et al. EFSA Panel on Food Additives and Nutrient Sources Added to Food. Scientific opinion on the safety of green tea catechins. EFSA Journal. 2018;16(4):e05239. DOI: 10.2903/j.efsa.2018.5239. [PubMed: 32625874]
12. Thompson M, Jaiswal Y, Wang I, Williams L. Hepatotoxicity: Treatment, causes and applications of medicinal plants as therapeutic agents. J Phytopharmacol. 2017;6(3):186–193. DOI: 10.31254/phyto.2017.6308.
13. Hoofnagle JH, Bonkovsky HL, Phillips EJ, et al. HLA-B*35:01 and Green Tea-Induced Liver Injury. Hepatol. 2021;73(6):2484–93. DOI: 10.1002/hep.31538.
14. Yu Z, Samavat H, Dostal AM, et al. Effect of Green Tea Supplements on Liver Enzyme Elevation: Results from a Randomized Intervention Study in the United States. Cancer Prev Res. 2017;10(10):571–579. DOI: 10.1158/1940-6207.CAPR-17-0160.
15. Lovera J, Ramos A, Devier D, et al. Polyphenon E, non-futile at neuroprotection in multiple sclerosis but unpredictably hepatotoxic: Phase I single group and phase II randomized placebo-controlled studies. J Neurol Sci. 2015;358(1–2):46–52. DOI: 10.1016/j.jns.2015.08.006. [PubMed: 26298797]
16. Miller RJ, Jackson KG, Dadd T, et al. A preliminary investigation of the impact of catechol-O-methyltransferase genotype on the absorption and metabolism of green tea catechins. Eur J Nutr. 2012;51(1):47–55. DOI: 10.1007/s00394-011-0189-0. [PubMed: 21445620]
17. González-Castro TB, Tovilla-Zárate C, Juárez-Rojop I, et al. Distribution of the Val108/158Met polymorphism of the COMT gene in healthy Mexican population. Gene. 2013;526(2):454–8. DOI: 10.1016/j.gene.2013.05.068. [PubMed: 23774690]
18. Shield AJ, Thomae BA, Eckloff BW, Wieben ED, Weinshilboum RM. Human catechol O-methyltransferase genetic variation: gene resequencing and functional characterization of

variant allozymes. Mol Psychiatry. 2004;9(2):151–60. DOI: 10.1038/sj.mp.4001386. [PubMed: 14966473]

19. Cai Z-Y, Li X-M, Liang J-P, et al. Bioavailability of Tea Catechins and Its Improvement. Molecules. 2018;23(9):2346. DOI: 10.3390/molecules23092346. [PubMed: 30217074]
20. Mohamed MEF, Frye RF. Inhibitory Effects of Commonly Used Herbal Extracts on UDP-Glucuronosyltransferase 1A4, 1A6, and 1A9 Enzyme Activities. Drug Metab Dispos. 2011;39(9):1522–1528. DOI: 10.1124/dmd.111.039602. [PubMed: 21632963]
21. Wang T, Cook I, Levh TS. Isozyme Specific Allosteric Regulation of Human Sulfotransferase 1A1. Biochemistry. 2016;55(29):4036–4046. DOI: 10.1021/acs.biochem.6b00401. [PubMed: 27356022]
22. McGill MR. The past and present of serum aminotransferases and the future of liver injury biomarkers. EXCLI J. 2016;15:817–828. DOI: 10.17179/excli2016-800. [PubMed: 28337112]
23. Kasarala G, Tillmann HL. Standard liver tests. Clin Liver Dis. 2016;8(1):13–18. DOI: 10.1002/cld.562.
24. Moriles KE, Azer SA. Alanine Amino Transferase. In: StatPearls. Treasure Island: StatPearls Publishing; 2022.
25. Gwaltney-Brant SM. Nutraceuticals in Hepatic Diseases. In: Gupta R, ed. Nutraceuticals: Efficacy, Safety and Toxicity. San Diego: Academic Press; 2016:87–99.
26. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. Toxicology. 2008;245(3):194–205. DOI: 10.1016/j.tox.2007.11.021. [PubMed: 18291570]
27. Oh RC, Hustead TR, Ali SM, Pantsari MW. Mildly Elevated Liver Transaminase Levels: Causes and Evaluation. Am Fam Physician. 2017;96(11):709–715. [PubMed: 29431403]
28. Vuppalanchi R, Chalasani N. Laboratory Tests in Liver Disease. In: Saxena R, ed. Practical Hepatic Pathology: A Diagnostic Approach. 2nd ed. Philadelphia: Elsevier; 2018:43–53.
29. Aragon G, Younossi ZM. When and how to evaluate mildly elevated liver enzymes in apparently healthy patients. Cleve Clin J Med. 2010;77(3):195–204. DOI: 10.3949/ccjm.77a.09064. [PubMed: 20200170]
30. Vilas-Boas V, Gijbels E, Jonckheer J, De Waele E, Vinken M. Cholestatic liver injury induced by food additives, dietary supplements and parenteral nutrition. Environ Int. 2020;136:105422. DOI: 10.1016/j.envint.2019.105422. [PubMed: 31884416]
31. Guy J, Peters MG. Liver disease in women: the influence of gender on epidemiology, natural history, and patient outcomes. Gastroenterol Hepatol. 2013;9(10):633–9.
32. Waxman DJ, Holloway MG. Sex differences in the expression of hepatic drug metabolizing enzymes. Mol Pharmacol. 2009;76(2):215–28. DOI: 10.1124/mol.109.056705. [PubMed: 19483103]
33. McLachlan AJ, Pont LG. Drug Metabolism in Older People—A Key Consideration in Achieving Optimal Outcomes With Medicines. J Gerontol. 2011;67A(2):175–180. DOI: 10.1093/gerona/glr118.
34. Brady CW. Liver disease in menopause. World J Gastroenterol. 2015;21(25):7613–20. DOI: 10.3748/wjg.v21.i25.7613. [PubMed: 26167064]
35. Samavat H, Dostal AM, Wang R, et al. The Minnesota Green Tea Trial (MGTT), a randomized controlled trial of the efficacy ofgreen tea extract on biomarkers of breast cancer risk: study rationale, design, methods,and participant characteristics. Cancer Causes Control. 2015;26(10):1405–1419. DOI: 10.1007/sl0552-015-0632-2. [PubMed: 26206423]
36. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39(2):175–191. DOI: 10.3758/bf03193146. [PubMed: 17695343]
37. Dostal AM, Samavat H, Bedell S, et al. The safety of green tea extract supplementation in postmenopausal women at risk for breast cancer: results of the Minnesota Green Tea Trial. Food Chem Toxicol. 2015;83:26–35. DOI: 10.1016/j.fct.2015.05.019. [PubMed: 26051348]
38. Kim HC, Nam CM, Jee SH, Han KH, Oh DK, Suh I. Normal serum aminotransferase concentration and risk of mortality from liver diseases: prospective cohort study. BMJ. 2004;328(7446):983–983. DOI: 10.1136/bmj.38050.593634.63. [PubMed: 15028636]

39. Prati D, Taioli E, Zanella A, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med. 2002;137(1):1–10. DOI: 10.7326/0003-4819-137-1-200207020-00006. [PubMed: 12093239]
40. De Ritis F, Coltorti M, Giusti G. An enzymic test for the diagnosis of viral hepatitis: The transaminase serum activities. Clinica Chimica Acta. 1957;2(1):70–74. DOI: 10.1016/0009-8981(57)90027-X.
41. Hall P, Cash J. What is the real function of the liver 'function' tests? Ulster Med J. 2012;81(1):30–6. [PubMed: 23536736]
42. Matsui S, Yasui T, Kasai K, et al. Changes of liver enzymes and triglyceride during the menopausal transition in Japanese women. J Obstet Gynaecol. 2016;36(6):806–811. DOI: 10.3109/01443615.2016.1154516. [PubMed: 27167882]
43. Srivastava K, Ochuba O, Sandhu JK, et al. Effect of Catechol-O-Methyltransferase Genotype Polymorphism on Neurological and Psychiatric Disorders: Progressing Towards Personalized Medicine. Cureus. 2021;13(9):e18311. DOI: 10.7759/cureus.18311. [PubMed: 34725583]
44. Acosta L, Radler DR, Kurzer M, Samavat H. The Effect of 12-Month Green Tea Extract Supplementation and Impact of Catechol-O-Methyltransferase Genotype on Blood Pressure in Women Who Are Post-menopause. Curr Dev Nutr. 2021;5(Suppl 2):291–291. DOI: 10.1093/cdn/nzab037_001.
45. Milenkovic D, Morand C, Cassidy A, et al. Interindividual Variability in Biomarkers of Cardiometabolic Health after Consuming of Major Plant-Food Bioactive Compounds and the Determinants Involved. Adv Nutr. 2017;8(4):558–570. DOI: 10.3945/an.116.013623. [PubMed: 28710143]
46. Morand C, Tomas-Barberan FA. Contribution of plant food bioactives in promoting health effects of plant foods: why look at interindividual variability? Eur J Nutr. 2019;58(Suppl 2):13–19. DOI: 10.1007/s00394-019-02096-0. [PubMed: 31637469]
47. Morand C, De Roos B, Garcia-Conesa MT, et al. Why interindividual variation in response to consumption of plant food bioactives matters for future personalised nutrition. Proc Nutr Soc. 2020;79(2):225–235. DOI: 10.1017/S0029665120000014. [PubMed: 32014077]
48. Shen CL, Chyu MC, Pence BC, et al. Green tea polyphenols supplementation and Tai Chi exercise for postmenopausal osteopenic women: safety and quality of life report. BMC Complement Altern Med. 2010;10:76. DOI: 10.1186/1472-6882-10-76. [PubMed: 21143878]
49. World Health Organization. Global Spending on Health: A World in Transition. Geneva: 2019. (https://www.who.int/publications/i/item/WHO-HIS-HGF-HFWorkingPaper-19.4). Accessed July 12, 2022.
50. Ojeda DA, López-León S, Forero DA. A novel cost-effective assay based on real-time PCR for COMT Val158Met genotyping. Biomarkers. 2014;19(7):567–70. DOI: 10.3109/1354750x.2014.948069. [PubMed: 25096984]

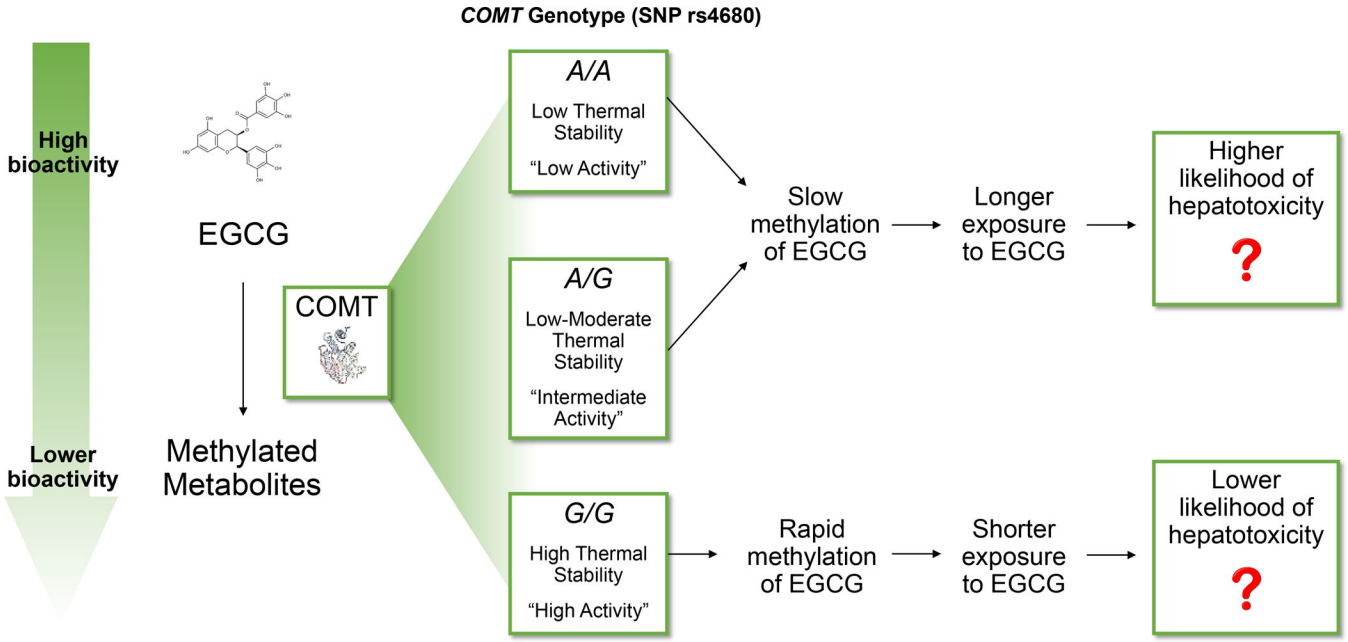

Figure 1:

Theoretical Basis for *Catechol-O-Methyltransferase* Genotype as a Predictor of Hepatotoxicity with Consumption of Green Tea Extract

Abbreviations: COMT, catechol-O-methyltransferase; EGCG, epigallocatechin gallate

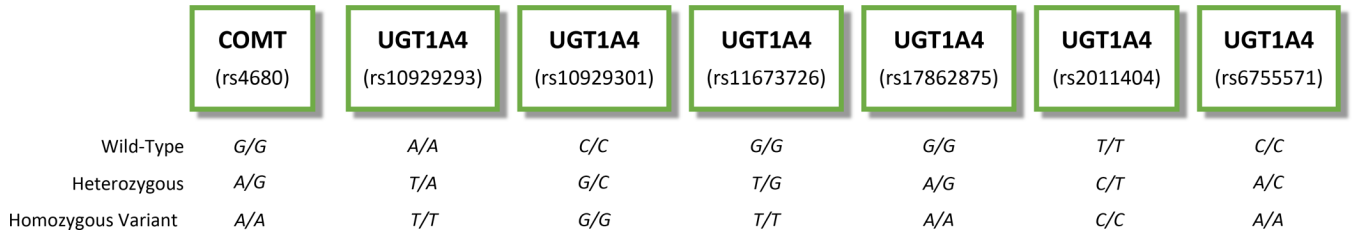

Figure 2:

Wild-type, Heterozygous, and Homozygous Variant Genotypes for Each SNP

Abbreviations: *COMT*, catechol-O-methyltransferase; SNP, single nucleotide polymorphism; *UGT1A4*, uridine 5′-diphospho-glucuronosyltransferase 1A4.

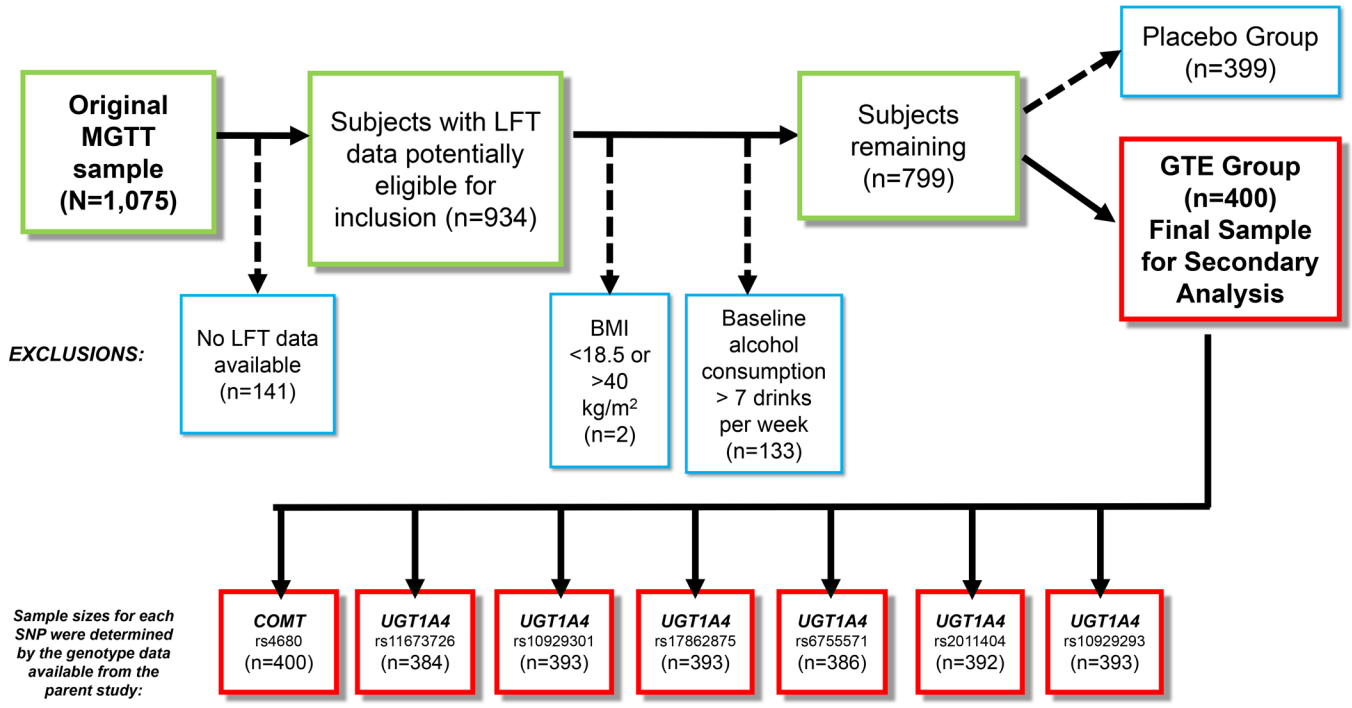

Figure 3:

Participant Flow Diagram for Determination of Final Sample

Abbreviations: *COMT*, catechol-O-methyltransferase; BMI, body mass index; kg, kilograms; LFT, liver function test; m, meters; MGTT, Minnesota Green Tea Trial; SNP, single nucleotide polymorphism; *UGT1A4*, uridine 5′-diphospho-glucuronosyltransferase 1A4.

Table 1:

Baseline Demographic, Lifestyle, Dietary, and Clinical Characteristics by *COMT* Genotype within the Green Tea Extract Group (n=400)

		***COMT* Genotype**			
Variable	**Total Sample in GTE Group (n=400)**	**High-activity (*G/G*) n=115**	**Intermediate-activity (*A/G*) n=156**	**Low-activity (*A/A*) n=129**	***P***
Age (years)	59.8 ± 4.8	59.9 ± 4.7	59.9 ± 4.7	59.7 ± 5.0	0.90[a]
BMI (kg/m^2)	25.1 ± 3.7	24.8 ± 3.8	25.5 ± 3.6	25.0 ± 3.8	0.30[a]
Race					0.35[b]
White	391 (97.8)	111 (96.5)	154 (98.7)	126 (97.7)	
Native American	3 (0.8)	1 (0.9)	0 (0.0)	2 (1.6)	
Asian	3 (0.8)	1 (0.9)	1 (0.6)	1 (0.8)	
Black	2 (0.5)	2 (1.7)	0 (0.0)	0 (0.0)	
Others	1 (0.3)	0 (0.0)	1 (0.6)	0 (0.0)	
Ethnicity					0.25[b]
Non-Hispanic	391 (97.8)	111 (96.5)	153 (98.1)	127 (98.4)	
Hispanic	4 (1.0)	3 (2.6)	0 (0.0)	1 (0.8)	
Unknown	5 (1.3)	1 (0.9)	3 (1.9)	1 (0.8)	
Herbal Supplement Use					0.34[b]
No	398 (99.5)	115 (100.0)	154 (98.7)	129 (100.0)	
Yes	2 (0.5)	0 (0.0)	2 (1.3)	0 (0.0)	
Medication Use					0.73[d]
No	31 (7.8)	7 (6.1)	13 (8.3)	11 (8.5)	
Yes	369 (92.3)	108 (93.9)	143 (91.7)	118 (91.5)	
Smoking Status					0.87[b]
Never	274 (68.5)	81 (70.4)	106 (67.9)	87 (67.4)	
Former	125 (31.3)	34 (29.6)	50 (32.1)	41 (31.8)	
Unknown	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.8)	
Dietary Intake					
Energy (kcal/d)	1419.4 ± 526.1	1447.8 ± 547.7	1365.4 ± 525.5	1459.4 ± 505.1	0.26[a]
Carbohydrate (% kcal)	51.3 ± 7.2	50.7 ± 8.2	51.6 ± 6.6	51.5 ± 6.9	0.54[a]
Protein (% kcal)	16.5 ± 2.8	16.5 ± 2.6	16.5 ± 3.0	16.5 ± 2.6	1.00[a]
Fat (% kcal)	33.2 ± 6.9	33.7 ± 7.9	32.9 ± 6.5	33.0 ± 6.3	0.63[a]
Saturated Fat (% kcal)	10.2 ± 2.3	10.2 ± 2.6	10.2 ± 2.3	10.2 ± 2.0	1.00[a]

		COMT Genotype			
Variable	**Total Sample in GTE Group (n=400)**	**High-activity (*G/G*) n=115**	**Intermediate-activity (*A/G*) n=156**	**Low-activity (*A/A*) n=129**	***P***
Alcohol (g/d)[†]	3.7 ± 3.7	4.2 ± 4.0	3.4 ± 3.6	3.6 ± 3.5	0.38[c]
Median (Q1, Q3)	2.4 (0.8, 5.7)	2.5 (0.9, 6.9)	2.0 (0.8, 5.6)	2.5 (0.6, 5.6)	

All continuous variables presented as mean ± SD unless otherwise noted. All categorical variables presented as n (%).

[†]Alcohol intake was non-normally distributed. Median (Q1, Q3) is provided in addition to mean ± SD.

[a]*P* values based on one-way analysis of variance (ANOVA).

[b]*P* values based on Fisher's exact test.

[c]*P* values based on Kruskal-Wallis test (K independent sample).

[d]*P* values based on chi-square test.

Abbreviations: AKP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; COMT, catechol-o-methyltransferase; GTE, green tea extract; SD, standard deviation.

Table 2:

Baseline Liver Enzyme Concentrations by *COMT* Genotype for Total Sample (N=799)

	***COMT* Genotype (rs4680)**					
Baseline Liver Enzymes	**Total Sample (N=799)**	**High-activity (*G/G*) n=215**	**Intermediate-activity (*A/G*) n=335**	**Low-activity (*A/A*) n=249**	***P unadjusted***	***P fully adjusted***
AST (U/L)	20.2 (19.9, 20.6)	20.6 (19.8, 21.3)	20.1 (19.6, 20.7)	20.0 (19.4, 20.7)	0.57	0.56
ALT (U/L)	17.8 (17.3, 18.3)	18.3 (17.4, 19.3)	17.7 (17.0, 18.4)	17.5 (16.6, 18.2)	0.38	0.39
AST: ALT Ratio	1.2 (1.2, 1.2)	1.2 (1.2, 1.3)	1.2 (1.2, 1.2)	1.2 (1.2, 1.3)	0.34	0.38
AKP (U/L)	69.9 (68.6, 71.2)	68.8 (66.4, 71.2)	70.5 (68.6, 72.5)	69.9 (67.7, 72.1)	0.34	0.53

Liver enzyme data are presented as adjusted means (95% confidence intervals).

P values (unadjusted) derived from one-way ANOVA.

P values (fully adjusted) derived from one-way ANCOVA adjusted for baseline age, BMI, ethnicity, smoking status, and intake of energy, carbohydrate, total fat, and alcohol.

Abbreviations: AKP, alkaline phosphatase; ALT, alanine aminotransferase; ANCOVA, analysis of covariance; ANOVA, analysis of variance; AST, aspartate aminotransferase; BMI, body mass index; COMT, catechol-o-methyltransferase.

Table 3:

Percent Liver Enzyme Changes by *COMT* Genotype within the Green Tea Extract Treatment Group (n=400)

	***COMT* Genotype (rs4680)**				
Liver Enzymes and Time Periods	**High-activity (*G/G*) n=115**	**Intermediate-activity (*A/G*) n=156**	**Low-activity (*A/A*) n=129**	***P unadjusted***	***P fully adjusted***
AST					
Baseline to Month 3	13.0 (−23.5, 49.6)	28.2 (−3.1, 59.6)	37.5 (3.1, 72.0)	0.63	0.63
Baseline to Month 6	20.1 (10.2, 30.1)	18.5 (9.9, 27.0)	15.2 (5.8, 24.6)	0.80	0.77
Baseline to Month 9	22.3 (11.4, 33.2)	20.7 (11.4, 30.1)	12.5 (2.2, 22.8)	0.38	0.37
Baseline to Month 12	20.4 (−17.2, 58.0)	45.2 (13.0, 77.4)	12.3 (−23.1, 47.7)	0.29	0.37
ALT					
Baseline to Month 3	22.6 (−47.8, 93.0)	45.1 (−15.3, 105.5)	82.3 (16.1, 148.5)	0.44	0.47
Baseline to Month 6	38.7 (21.2, 56.1)	26.9 (11.9, 41.9)	35.1 (18.7, 51.5)	0.73	0.58
Baseline to Month 9	46.0 (25.2, 66.7)	35.1 (17.3, 53.0)	26.4 (6.8, 46.0)	0.46	0.41
Baseline to Month 12	40.2 (−44.7, 125.1)	88.3 (15.5, 161.2)	23.6 (−56.3, 103.5)	0.37	0.47
AST: ALT Ratio					
Baseline to Month 3	-3.3^{ab} (−6.7, 0.1)	1.0^{a} (−1.9, 3.9)	-4.8^{b} (−8.0, −1.6)	**0.05**	**0.03**
Baseline to Month 6	−4.1 (−7.9, −0.3)	−0.2 (−3.5, 3.0)	−4.2 (−7.7, −0.6)	0.31	0.19
Baseline to Month 9	−4.6 (−8.5, −0.7)	−3.1 (−6.4, 0.3)	−3.2 (−6.9, 0.5)	0.91	0.83
Baseline to Month 12	−3.0 (−7.3, 1.2)	0.8 (−2.9, 4.4)	−0.9 (−4.9, 3.1)	0.72	0.42
AKP					
Baseline to Month 3	1.6 (−1.0, 4.2)	1.0 (−1.2, 3.3)	2.5 (0.0, 4.9)	0.60	0.70
Baseline to Month 6	1.6 (−0.7, 4.0)	−0.8 (−2.8, 1.2)	0.2 (−2.0, 2.4)	0.31	0.30
Baseline to Month 9	3.4 (0.8, 6.0)	3.1 (0.9, 5.4)	1.6 (−0.8, 4.1)	0.50	0.55
Baseline to Month 12	2.5 (0.2, 4.9)	0.3 (−1.7, 2.3)	0.6 (−1.6, 2.9)	0.33	0.33

Values are presented as adjusted means (95% confidence intervals).

Bolded *p* values indicate significance at $P \leq 0.05$.

P values (unadjusted) are derived from one-way ANOVA.

P values (fully adjusted) are derived from one-way ANCOVA adjusted for baseline age, BMI, ethnicity, smoking status, and intake of energy, carbohydrate, total fat, and alcohol.

[a,b] Values with differing letters as a superscript are significantly different at $P \leq 0.05$ adjusted for multiple comparisons using Bonferroni's post-hoc test.

Abbreviations: AKP, alkaline phosphatase; ALT, alanine aminotransferase; ANCOVA, analysis of covariance; ANOVA, analysis of variance; AST, aspartate aminotransferase; COMT, catechol-o-methyltransferase.

Table 4:

Mean Liver Enzyme Concentrations by *COMT* Genotype at Baseline and Months 3, 6, 9, and 12 within the Green Tea Extract Treatment Group (n=400)

	***COMT* Genotype (rs4680)**			
Liver Enzymes and Time Points	**High-activity (G/G) n=115**	**Intermediate-activity (A/G) n=156**	**Low-activity (A/A) n=129**	***P interaction***
AST (U/L)				0.72
Baseline	20.2[a] (19.2, 21.3)	19.7[a] (19.0, 20.5)	19.6[a] (18.8, 20.4)	
Month 3	22.1[ab] (20.6, 23.7)	24.7[ab] (20.0, 29.5)	27.1[ab] (16.4, 37.7)	
Month 6	23.3[b] (21.5, 25.1)	23.3[b] (21.3, 25.2)	22.1[b] (20.7, 23.6)	
Month 9	23.6[ab] (21.4, 25.9)	23.5[b] (21.6, 25.3)	21.6[b] (20.3, 22.9)	
Month 12	23.1[ab] (21.2, 24.9)	27.8[ab] (18.9, 36.8)	21.5[b] (20.3, 22.7)	
P value	**0.02**	**<0.001**	**0.004**	
ALT (U/L)				0.62
Baseline	18.3[a] (16.8, 19.8)	17.7[a] (16.6, 18.7)	16.9[a] (15.8, 18.0)	
Month 3	21.6[ab] (18.8, 24.5)	24.4[ab] (17.4, 31.4)	28.3[ab] (14.4, 42.3)	
Month 6	23.4[b] (20.3, 26.4)	22.5[b] (19.5, 25.5)	21.4[b] (19.0, 23.7)	
Month 9	24.7[b] (20.4, 29.0)	23.3[b] (20.4, 26.2)	20.1[b] (18.3, 22.0)	
Month 12	22.8[b] (19.8, 25.7)	29.1[ab] (16.8, 41.4)	19.5[b] (17.8, 21.2)	
P value	**0.02**	**<0.001**	**0.004**	
AST: ALT Ratio				0.26
Baseline	1.21[a] (1.14, 1.28)	1.18[a] (1.14, 1.22)	1.25[a] (1.19, 1.30)	
Month 3	1.16[ab] (1.09, 1.23)	1.17[ab] (1.13, 1.21)	1.16[b] (1.10, 1.22)	
Month 6	1.13[b] (1.07, 1.19)	1.16[ab] (1.11, 1.20)	1.16[b] (1.11, 1.21)	
Month 9	1.13[b] (1.06, 1.19)	1.12[b] (1.08, 1.16)	1.17[b] (1.12, 1.23)	
Month 12	1.14[ab] (1.09, 1.20)	1.16[ab] (1.11, 1.20)	1.19[ab] (1.14, 1.24)	
P value	**0.04**	**0.03**	**0.01**	
AKP (U/L)				0.36
Baseline	66.4 (63.4, 69.4)	72.0[ab] (68.8, 75.3)	68.0 (65.0, 71.0)	
Month 3	66.7 (63.9, 69.4)	72.2[ab] (68.6, 75.7)	69.1 (65.9, 72.3)	
Month 6	67.0 (63.8, 70.3)	70.7[a] (67.7, 73.7)	67.5 (64.6, 70.4)	
Month 9	68.1 (65.1, 71.2)	73.4[b] (70.1, 76.7)	68.4 (65.6, 71.2)	
Month 12	67.5 (64.3, 70.7)	71.4[ab] (68.4, 74.4)	67.7 (64.9, 70.5)	
P value	0.24	**0.01**	0.38	

Liver enzyme data by *COMT* genotype are presented as adjusted means with 95% confidence intervals. Means are adjusted for baseline age, BMI, ethnicity, smoking status, and intake of energy, carbohydrate, total fat, and alcohol.

Bolded *P* values indicate significance at $P \leq 0.05$.

$P_{Interaction}$ values are based on general linear model and Wilks' Lambda.

P values within each *COMT* genotype are based on Wilks' Lambda with repeated measures ANOVA, adjusted for baseline age, BMI, ethnicity, smoking status, and intake of energy, carbohydrate, total fat, and alcohol.

^{a,b}Values with differing letters as a superscript are significantly different at $P \leq 0.05$ adjusted for multiple comparisons using Bonferroni's post-hoc test.

Abbreviations: AKP, alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BMI, body mass index; COMT, catechol-o-methyltransferase.

Table 5:

Percent Liver Enzyme Changes by *UGT1A4 (rs6755571)* Genotype within the Green Tea Extract Treatment Group (n=386)

	***UGT1A4* Genotype (rs6755571)**			
Time Periods	**Wild-Type (C/C) n=339**	**Heterozygous (A/C) n=47**	***P unadjusted***	***P fully adjusted***
AST				
Baseline to Month 3	27.5 (5.9, 49.1)	28.4 (−30.0, 86.7)	0.97	1.00
Baseline to Month 6	15.9 (10.1, 21.7)	36.3 (20.6, 52.1)	**0.01**	**0.02**
Baseline to Month 9	15.8 (9.5, 22.2)	41.5 (24.3, 58.7)	**0.01**	**0.01**
Baseline to Month 12	29.1 (6.9, 51.3)	22.5 (−37.6, 82.5)	0.87	0.84
ALT				
Baseline to Month 3	48.6 (7.1, 90.2)	77.2 (−35.1, 189.6)	0.61	0.64
Baseline to Month 6	28.0 (17.9, 38.1)	78.1 (50.8, 105.4)	**<0.001**	**<0.001**
Baseline to Month 9	30.1 (18.0, 42.3)	82.1 (49.3, 114.9)	**0.003**	**0.004**
Baseline to Month 12	58.0 (7.8, 108.1)	35.1 (−100.6, 170.7)	0.81	0.76
AST: ALT Ratio				
Baseline to Month 3	−1.7 (−3.7, 0.3)	−5.8 (−11.1, −0.5)	0.06	0.15
Baseline to Month 6	−2.4 (−4.6, 0.2)	−7.0 (−12.9, −1.1)	0.06	0.16
Baseline to Month 9	−3.5 (−5.7, −1.2)	−6.0 (−12.1, 0.1)	0.26	0.45
Baseline to Month 12	−1.3 (−3.7, 1.2)	0.2 (−6.5, 6.8)	0.84	0.69
AKP				
Baseline to Month 3	2.1 (0.6, 3.6)	1.4 (−2.6, 5.5)	0.62	0.77
Baseline to Month 6	0.3 (−1.1, 1.7)	2.7 (−0.9, 6.4)	0.21	0.22
Baseline to Month 9	2.9 (1.4, 4.4)	4.2 (0.1, 8.3)	0.60	0.55
Baseline to Month 12	1.5 (0.2, 2.8)	0.3 (−3.3, 3.9)	0.52	0.55

Note: The *A/A* genotype was excluded from these analyses due to small sample size (n=1).

Liver enzyme data by *UGT1A4 (rs6755571)* genotype are presented as adjusted means with 95% confidence intervals.

Bolded *P* values indicate significance at $P \leq 0.05$.

P values (unadjusted) are derived from one-way ANOVA.

P values (fully adjusted) are derived from one-way ANCOVA adjusted for baseline age, BMI, ethnicity, smoking status, and intake of energy, carbohydrate, total fat, and alcohol.

Abbreviations: AKP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; UGT1A4, uridine 5′-diphospho-glucuronosyltransferase 1A4.