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RESEARCH ARTICLE

## Gene-by-Environment Interaction in Non-Alcoholic Fatty Liver Disease and Depression: The Role of Hepatic Transaminases

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### ABSTRACT:

Non-alcoholic fatty liver disease (NAFLD) encompasses a range of liver conditions, from benign fatty accumulation to severe fibrosis. The global prevalence of NAFLD has risen to 25-30%, with variations across ethnic groups. NAFLD may advance to hepatocellular carcinoma, increases cardiovascular risk, is associated with chronic kidney disease, and is an independent metabolic disease risk factor. Assessment methods for liver health include liver biopsy, magnetic resonance imaging, ultrasound, and vibration-controlled transient elastography (VCTE by FibroScan). Hepatic transaminases are cost-effective and minimally invasive liver health assessment methods options.

This study focuses on the interaction between genetic factors underlying the traits (hepatic transaminases and the FibroScan results) on the one hand and the environment (depression) on the other. We examined 525 individuals at risk for metabolic disorders. We utilized variance components models and likelihood-based statistical inference to examine potential GxE interactions in markers of NAFLD, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and the AST/ALT ratio, and Vibration-Controlled Transient Elastography (VCTE by FibroScan). We calculated the Fibroscan-AST (FAST) score (a score that identifies the risk of progressive non-alcoholic steatohepatitis (NASH) and screened for depression using the Beck Depression Inventory-II (BDI-II). We identified significant G x E interactions for AST/ALT ratio x BDI-II, but not AST, ALT, or the FAST score. Our findings support that genetic factors play a role in hepatic transaminases, especially the AST/ALT ratio, with depression influencing this relationship. These insights contribute to understanding the complex interplay of genetics, environment, and liver health, potentially guiding future personalized interventions.

**Keywords:** Mexican Americans, GxE, Transaminases, FAST Score, De Ritis ratio, FibroScan, Genetics, Heritability, Depression

## Introduction

The hepatic histologic spectrum of non-alcoholic fatty liver disease (NAFLD) ranges from non-alcoholic fatty liver to non-alcoholic steatohepatitis, advanced fibrosis, and cirrhosis.<sup>1</sup> The prevalence of NAFLD has risen to a global population high of 25-30%, with significant variation among ethnic groups.<sup>2-5</sup> NAFLD is a significant health concern that can progress to hepatocellular carcinoma<sup>5</sup>, increases cardiovascular risk, is associated with higher rates of chronic kidney disease, and is an independent risk factor for system-wide metabolic disease.<sup>6,7</sup>

Hepatic transaminases are often used for screening as they are less invasive and more cost-effective than advanced technology.<sup>8</sup> Increased aspartate aminotransferase (AST), alanine aminotransferase (ALT), and the AST/ALT (Di Ritis)<sup>9</sup> ratio are the common laboratory measures used in the evaluation of liver health (AST > 37 or ALT > 40 U/L in males or AST or ALT > 31 U/L in females and an AST/ALT ratio > 1). High concentrations of ratio are associated with various conditions, including NAFLD--the most common cause of elevated liver enzymes in adults in the United States, Japan, Australia, Europe, and the Middle East.<sup>10</sup> A strong association exists between metabolic syndrome (central obesity, hypercholesterolemia, reduced HDL-cholesterol levels, hypertension, and elevated plasma triglycerides, with insulin resistance) and the AST/ALT ratio.<sup>11</sup>

Other measures of liver health include biopsy (invasive) and Magnetic Resonance Imaging (expensive), as well as Vibration Controlled Transient Elastography (VCTE by FibroScan)<sup>2,8</sup> which is accurate and facilitates liver health measurement in community-based healthcare and research settings.<sup>12,13</sup> FibroScan quantifies the speed of the shear wave propagated by the ultrasonic wave through the liver. The controlled attenuation parameter (CAP) measures liver ultrasonic attenuation (degree of steatosis). A CAP of 300 dB/m is an accurate cutoff (PPV 95% CI) and NPV (95% CI) for diagnosing fatty infiltration. Liver stiffness measurements (LSMs) are expressed in kilopascals (kPa) and accurately measure the level of fibrosis.<sup>14</sup> The presence of NAFLD is determined based on VCTE results and hepatic transaminases (FAST-AST) or FAST Score.<sup>8</sup>

In our previous publication, we provide evidence that genetic factors interact with depression to influence the expression of hepatic fibrosis in Mexican Americans in our region.<sup>15</sup> Plasma concentrations of liver enzymes are highly heritable (20-77%),<sup>16</sup> suggesting a genetic role that may

help interpret results and explain variation among individuals. Our findings shed light on the complex interplay between genetic predisposition, environmental factors, and mental health in the context of NAFLD among Mexican Americans. This study aims to determine if there are interactions between the genetic factors underlying the traits (hepatic transaminases and the FibroScan) on the one hand and the environment (depression).

## Materials and Methods

The University of Texas Rio Grande Valley IRB approved the study protocol. All participants provided informed consent before participating in the study. In an ongoing genetic epidemiological study, we evaluated 525 Mexican American participants recruited from the community at risk for obesity, diabetes, hypertension, hyperlipidemia, and depression. The Rio Grande Valley is predominantly Mexican American (90 percent) with disproportionately high rates of obesity (55.5%), diabetes (32.5%), and depression (19%).<sup>15</sup> Information gathered included biometric data, an assessment of depression (BDI-II), and transaminases (AST, ALT, AST/ALT ratio). We also measured FibroScan results. The controlled attenuation parameter (CAP) measures liver ultrasonic attenuation, measuring the degree of steatosis. We calculated the FibroScan-AST (FAST) score, which identifies the risk of progressive non-alcoholic steatohepatitis (NASH) (positive predictive value (PPV) of 0.83 and a negative predictive value (NPV) of 0.85).<sup>8</sup> Inclusion criteria included age of 18 years or older, residence in the Rio Grande Valley, and having four grandparents who are either Mexican or Mexican American.

The Beck Depression Inventory-II (BDI-II) was used to assess the degree of depressive symptoms over two weeks.<sup>17</sup> The BDI-II assesses the severity of depression and is an acceptable screening instrument for depression when administered in both Spanish and English.<sup>17-20</sup>

## STATISTICAL ANALYSIS

To test for gender differences in the variables, we used the non-parametric Mann-Whitney-Wilcoxon test which is robust to non-normality in the data. We estimated heritabilities ( $h^2$ ) and genotype x environment interaction using a variance component approach as implemented in the computer program SOLAR. <http://solar-eclipse-genetics.org/brief-overview.html>. Each liver-related phenotype (AST, ALT, AST/ALT, and FAST) was regressed against age, sex, age-squared, sex-by-age, and sex-by-age-squared, and then the regression residuals derived for each trait were normalized using an inverse normal transformation.<sup>21</sup>

## GENOTYPE-BY ENVIRONMENT (G X E) INTERACTION MODEL FOR CONTINUOUS ENVIRONMENTS

The base model—known as the polygenic model—is used to obtain estimates of liver trait heritabilities and as a model reference point upon which complex models can be elaborated. For a sample of related individuals, the polygenic model posits that the phenotypic covariance is decomposable into additive genetic and residual environmental variance components, and that inter-individual covariances will be given strictly by the additive genetic variance weighted by the genetic relatedness coefficient, assuming (for genetic covariance) that the pairwise genetic correlation across environments is unity, and that the additive genetic variance is homogeneous. Under the  $G \times E$  model, we relax these assumptions by expressing both the additive genetic variance and genetic correlations as continuous functions of a specific environment (e.g., extent of depressive symptoms) to capture any potential interaction between the genetic effects (i.e., the additive genetic variance and/or genetic correlation) and the specific environment. The null hypothesis is that the expression of the aggregate of all genotypes underlying a phenotype (polygenotype) is independent of the specific environment. Rejection of the null hypothesis implies that the genotype-phenotype map for the trait in question depends on a specific environment or is a function of the specific environment. We begin to study the problem of the genotype-phenotype map potentially being dependent on the environment by modeling the GxE interaction variance. The GxE interaction variance is zero if the following two conditions are simultaneously true: (1) homogeneity of the additive genetic variance across environments:  $\sigma_{g1}^2 = \sigma_{g2}^2 = \sigma_{g}^2$ , where  $\sigma_{g1}^2$  and  $\sigma_{g2}^2$  are the additive genetic variance in environments 1 and 2, respectively; (2) complete pleiotropy (i.e., the same genes are active across environments) in which the genetic correlation ( $\rho_g$ ) is one across environments:  $\rho_g = 1$ . There is evidence of GxE if either null hypothesis is rejected.<sup>22</sup> Rejection of either or both is evidence that the *phenotypic response to the environment* has a genetic basis.

We modeled the genetic variance and cross-environment genetic correlation as functions of depression, where the quantitative measure of depression is given as the total score on the BDI-II. Since it is likely that our focal environment is also influenced by genetic factors, we first tested for genetic factors underlying the BDI-II measure of depression and observed a significant heritability of 0.38 ( $p < 1.0 \times 10^{-5}$ ). Because we are interested in

the purely environmental component of depression, we computed a prediction of the associated genetic values using Best Linear Unbiased Prediction (BLUP) methods. BLUP accounts for additive genetic and environmental covariances among relatives based on known pedigree structure.<sup>23</sup> We then subtracted the BLUP genetic values for BDI from the original (BDI-derived) depression variable to get a BLUP-computed depression variable that reflects primarily environmental effects.<sup>23</sup> This lattermost variable is the focal (genetically corrected) environment in our GxE model.

For the genetic variance function (and similarly for the environmental variance), we modeled the variance using an exponential function of depression, where the exponential function maintains positivity, which is required of a variance<sup>24</sup> ( $\sigma_g^2 = \exp[\alpha_g + \gamma_g(\text{BDI})]$ ), where  $\alpha_g$  and  $\gamma_g$  are parameters to be estimated. Taking the natural logarithm of the exponential function, the variance homogeneity null hypothesis holds for a slope-term equal to 0:  $\gamma_g = 0$ . The genetic correlation was modeled using the exponential decay function of the pairwise differences in BDI scores:  $\rho_g = \exp[-\lambda|\text{BDI}_x - \text{BDI}_z|]$  where  $\text{BDI}_x$  and  $\text{BDI}_z$  are the values of the BDI for any two individuals  $x$  and  $z$ . The null hypothesis that the genetic correlation is equal to 1 is equivalent to  $\lambda = 0$  because in this event:  $\rho_g = \exp[-\lambda|\text{BDI}_x - \text{BDI}_z|] = e^0 = 1$ .

We carried out model evaluations and hypothesis testing in two stages. In stage one, we examined if the overall  $G \times E$  interaction model provided a better fit to the data when compared with the polygenic model by way of a likelihood ratio test (LRT). It is important to note that the polygenic model is fully nested within the  $G \times E$  interaction model and that relative to the polygenic model, the  $G \times E$  interaction model has three additional parameters ( $\gamma_g$ ,  $\gamma_e$ , and  $\lambda$ ;  $\alpha_g$  and  $\alpha_e$  are re-parameterized versions of the variances). The LRT statistic for this comparison is distributed as a 50:50 mixture of chi-squares with 2 and 3 degrees of freedom (df).<sup>21,22,25</sup>

In the second stage, we examine the more specific  $G \times E$  interaction hypotheses. The full  $G \times E$  model with all parameters estimated was compared with models when either gamma ( $\gamma$ ) or lambda ( $\lambda$ ) was constrained to 0 to respectively test the hypotheses of additive genetic variance homogeneity and a genetic correlation equal to one. The distributions of the LRT statistics are, respectively, a chi-square with 1 df, and a 50:50 mixture of a chi-square with a point mass at 0 and a chi-square with 1 df.<sup>15,21</sup> As

part of this stage, we determined if each of the three additional parameters in the full  $G \times E$  interaction model ( $\gamma_g$ ,  $\gamma_e$ , and  $\lambda$ ) should even be included at all by comparing its maximum likelihood estimate (MLE) to its standard error (SE). A parameter is roughly significant if its MLE is greater than twice its SE based on likelihood theory. Therefore, if a parameter SE was greater than its MLE, we judged that parameter to be statistically unimportant. Further, the additional parameters were formally tested by the tests mentioned above. If any of the three additional parameters were found to have SEs greater than their MLEs and if

these were found to be formally insignificant, we then compared a reduced version of the  $G \times E$  interaction model to the polygenic model, excluding the insignificant parameters.

## Results

The demographic characteristics (age, AST, ALT, AST/ALT ratio, and FAST, BDI-II) by sex of the cohort are listed in Table 1. There were no significant differences between age for males and females, but there are differences for AST, ALT, AST/ALT ratio, and FAST scores as inferred from the Mann-Whitney Wilcoxon tests.

**Table 1:** Demographic characteristics of the sample

Trait	Females N=391		Males N=134	
	Mean	SD	Mean	SD
Age	44.33	14.76	45.96	15.70
AST	19.88	15.99	25.67	19.99
ALT	22.18	24.05	33.00	31.53
AST/ALT Ratio	1.17	0.66	1.00	0.63
FAST	0.13	0.19	0.18	0.21
BDI-II	6.99	8.25	4.64	6.60

All 6 variables were tested for differences across AST, ALT, AST/ALT ratio, and FAST scores as inferred from the Mann-Whitney Wilcoxon tests.

## HERITABILITY

Data from 525 individuals were analyzed. As reported in Table 2, we found statistically significant moderate heritabilities for AST ( $h^2 = .25$ ,  $p = 0.03$ ), ALT ( $h^2 = 0.41$ ,  $p = 8.1E-04$ ), AST/ALT ( $h^2$

$= 0.26$ ;  $p = 0.004$ ), and FAST ( $h^2 = 0.36$ ;  $p = 1.5E-03$ ), and BDI ( $h^2 = .37$ ;  $p = 7.8E-06$ ). We formally compared the full  $G \times E$  interaction model to the polygenic model for AST, ALT, AST/ALT ratio, and FAST score (Table 3).

**Table 2:** Heritability analysis of transaminases and FAST score variables

Trait	Heritability	Standard Error	Sample Size	p-value
AST	0.25	0.14	525	0.03
ALT	0.41	0.13	525	8.1E-04
AST/ALT Ratio	0.26	0.10	525	.004
FAST	0.36	0.12	475	.15E-03
BDI-II	0.37	0.10	525	7.8E-06

**Table 3:** Testing the full  $G \times E$  interaction model against the polygenic model

Trait	Model	Ln likelihood	Chi-square	p-value
AST	Polygenic	-255.29	1.68	0.54
	Full GxE	-254.45		
ALT	Polygenic	-252.41	1.92	0.49
	Full GxE	-251.45		
AST/ALT	Polygenic	-253.92	16.19	6.7E-4
	Full GxE	-245.82		
FAST	Polygenic	-138.96	2.30	0.41
	Full GxE	-137.81		

It is important to note that full  $G \times E$  interaction model has three additional parameters of interest compared to the basal polygenic model, one of which has a null hypothesis on the boundary of its

permissible parameter space. For this reason, the formal comparison gives a 50:50 mixture of chi-squares with 2 and 3 degrees of freedom (df). To ensure best-model selection, we took under

consideration that the two slope parameters, which allow for genetic and environmental variance heterogeneity, both had standard errors larger than their respective maximum likelihood estimates (MLEs), whereas the MLE for the genetic correlation decay parameter was larger than its standard error.

Formal 1 df testing of the genetic and environmental variance heterogeneity parameters showed that AST, ALT, and FAST were not significant. Results for AST/ALT ratio are demonstrated in Table 4. The additive genetic variance decreased with an increased BDII-II environment.

**Table 4:** Testing the critical parameters of the full  $G \times E$  interaction model for AST/ALT

Model	Ln likelihood	Chi-square	p-value
Constrained genetic slope	<b>-248.02</b>	<b>4.40</b>	<b>0.04</b>
Constrained environmental slope	<b>-246.96</b>	<b>2.28</b>	<b>0.13</b>
Constrained genetic correlation decay	<b>-250.18</b>	<b>0.00</b>	<b>0.50</b>
Full $G \times E$ interaction model	<b>-245.82</b>	<b>N/A</b>	<b>N/A</b>

## Discussion

Mexican Americans are carriers of chronic disease risk alleles, including obesity, NAFLD, diabetes, and cardiovascular disease.<sup>15,26,27</sup> There is evidence of an interaction between genes and NAFLD in Mexican Americans.<sup>15</sup> This study adds several key additional contributions. Firstly, this is a large, robust to population stratification, family-based design, with the ability to perform genetic analyses that cannot be accomplished with a sample of unrelated individuals.<sup>28</sup> Using variance component models, likelihood-based statistical inference, and a BLUP-computed depression model, we identified significant GxE interaction variance. We also confirmed that the AST/ALT ratio and the BDI-II are heritable,<sup>29-35</sup> and discovered a new finding that the FAST score is also moderately heritable. Depression influences genes underlying the expression of the AST/ALT ratio. Notably, there is a decrease in additive genetic variance with higher AST/ALT levels, suggesting a decrease in GXE interaction with increased depression measured by BDI-II.

The role of depression in the pathophysiology of liver disease garners significant attention.<sup>36</sup> Using a meta-analysis, Xiao et al. demonstrated that patients with non-alcoholic steatohepatitis have a significantly higher prevalence of depression than patients with NAFLD (RR: 2.83,  $p < 0.001$ ), and there is a shared association between both conditions.<sup>37</sup> Risk factors for NAFLD include obesity, diabetes, hyperlipidemia, and female gender.<sup>38,39</sup> We found consistent evidence of GxE effects for AST/ALT ratio and depression, commensurate with previous findings that the variation in hepatic transaminases is, in part, genetically determined.<sup>31,32,35</sup> Sutoh et al. found that alcohol increased the AST/ALT ratio through reduced ALT levels without AST change in middle-aged Japanese men with the ALDH2 genotype.<sup>40</sup> ALT is especially useful as a clinical laboratory marker

associated with drug hepatotoxicity, hepatocellular death, and fatty degeneration. ALT can be influenced by age, sex, dietary change, geographical location, ethnicity, obesity, and marital status. Human isozymes are located in the cytosol and mitochondria. Mitochondrial ALT is not present in normal human serum and is the most important indicator of hepatocellular injury.<sup>41</sup> AST is found in the cytosol and mitochondria of hepatocytes, but also in skeletal muscle, kidney, and pancreas. Approximately 80% of 60-70% of AST activity in hepatocytes is in the mitochondria. AST is an indicator of mitochondrial damage.<sup>41</sup>

The hepatic transaminases, especially the AST/ALT ratio, are widely used to evaluate the risk of fatty infiltration and fibrosis.<sup>42,43</sup> Why there is a gene-by-environment interaction between genes underlying the AST/ALT ratio that is influenced by (decreased additive variance) with increased depression) could be related to genes underlying the cellular sources of AST and ALT. The underlying genetic influence on AST and ALT may affect different cellular processes. Clinicians use measures of liver health to help identify risk, disease, and etiology. Our findings support an underlying genetic influence on transaminases that theoretically vary according to impact on cellular physiology.

Increased expression of inflammatory pathways may explain the NAFLD-depression interaction.<sup>44,45</sup> Growing evidence supports NAFLD as a metabolic companion of psychiatric disorders with common shared inflammatory pathways.<sup>46-50</sup> NAFLD and depression are involved in a complex system with shared pathogenesis mediated by inflammatory dysregulation, oxidative stress pathways, and mitochondrial dysfunction (metabolic and-inflammatory effectors).<sup>49,50</sup> Our team is investigating which genetic transcripts are involved in NAFLD in the face of depression. The results will provide more information regarding the role of

inflammation at the cellular level, how clinical measures of liver health such as VCTE, FAST score, and hepatic transaminase results are related to liver health, and how the environment modulates the underlying genetic response.

### Implications for Research

Measurement of transaminases and the FibroScan-AST score are commonly used to diagnose and determine the progression of NAFLD. The determinants of decreased reliability of Liver stiffness measurement (LSM) include older age, obesity, higher liver stiffness, and operator experience, which may contribute to the results of the FAST score. Our findings show that a set of genes influences the expression of the AST/ALT ratio and not the individual transaminases or the FAST score. The AST/ALT ratio may be a more sensitive test than the individual transaminases, and therefore there is more power to detect differences (effect size), or our population may have varying exposure to liver toxins. Our focus is on Mexican Americans, and future research will determine if the finding of genotype by environment interaction

effects between NAFLD and depression is replicated in other populations.

### Conclusions

We examined potential G x E interaction using variance component models and likelihood-based statistical inference in the phenotypic expression of NAFLD, focusing on liver transaminases and the FAST score. Clinicians routinely use both measures, and are cost-effective and non-invasive. We assessed depression (environment) using the Beck Depression Inventory-II and identified significant G x E interactions of AST/ALT in response to depression. We confirmed earlier reports of the heritability of hepatic transaminases and the BDI-II in Mexican Americans. We also determined the heritability of the FAST score. We are currently investigating the nature of the interactions and the specific genes involved.

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