

# Copy Number Variation in the *GSTM1* and *GSTT1* Genes and the Risk of Liver Cirrhosis in Eastern Ethiopia

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**Background:** Polymorphisms in glutathione S-transferase M1 (*GSTM1*) and T1 (*GSTT1*) can cause an entire gene deletion. The current methodology can accurately identify *GSTM1* and *GSTT1* copy number variants (CNVs), which may shed light on the true contribution of each gene copy to the cellular detoxification process and disease risk. Because liver cirrhosis is becoming a critical worldwide health issue, this study determined the CNVs of *GSTM1* and *GSTT1* and their relationship to the risk of liver cirrhosis.

**Methods:** In this study, we compared 106 patients with liver cirrhosis to 104 healthy controls. Real-time PCR was used to identify the CNVs of *GSTM1* and *GSTT1*. Logistic and linear regression models were used to estimate the relationship between liver cirrhosis and clinical chemistry variables with the CNVs, respectively.

**Results:** In 3.3% of the study participants, >2 copies of the *GSTM1* or *GSTT1* genes were detected. *GSTT1* carriers had a significantly lower risk of liver cirrhosis ( $p < 0.05$ ) compared with individuals who had homozygous deletion (adjusted odds ratio (AOR) = 0.47; 95% CI: 0.25, 0.86). This risk reduction was significant ( $p < 0.05$ ) in patients with a single copy of the *GSTT1* gene (AOR = 0.48; 95% CI: 0.25, 0.91). Those with  $\geq 2$  copies of combined *GSTM1* and *GSTT1* also had a significantly ( $p < 0.05$ ) lower risk of developing liver cirrhosis compared with double null genotypes (AOR = 0.38; 95% CI: 0.16, 0.91,  $p$  trend < 0.001). Moreover,  $\geq 2$  copies of combined *GSTM1* and *GSTT1* genes were associated with a substantial decrease in alanine amino transferase (ALT) and aspartate aminotransferase (AST) levels, respectively.

**Conclusion:** A single copy number of *GSTT1*, and  $\geq 2$  copies of combined *GSTM1* and *GSTT1* genes were associated with a reduced risk of liver cirrhosis in Ethiopians. These findings underscore the importance of gene–environment interactions in the multifactorial development of liver cirrhosis.

**Keywords:** liver cirrhosis, copy number variation, glutathione S-transferase genes, Ethiopia

## Introduction

Glutathione S-transferase (GST) enzymes are generally known to protect cells from oxidative stress due to metabolic products of endogenous and exogenous chemicals.<sup>1</sup> GST's biochemical defense mechanisms include both the reduction of organic hydroperoxides, which contribute to oxidative stress, and the conjugation of electrophilic chemicals with glutathione, which facilitates their transport out of the cell.<sup>2,3</sup> There are 16 genes coding for the cytosolic form of GST enzymes in humans and are classified into 7 classes: alpha (*GSTA*), mu (*GSTM*), pi (*GSTP*), theta (*GSTT*), omega (*GSTO*), and zeta (*GSTZ*).<sup>1</sup> These genes are known to be highly polymorphic,<sup>4</sup> which can result in altered detoxification and oxidative stress defense capacity in certain tissues, particularly the liver, which has a central metabolic role.<sup>5,6</sup>

Oxidative stress is thought to be the primary cause of liver injury and progression to liver cirrhosis as well as hepatocellular carcinoma (HCC) associated with aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) exposure.<sup>7,8</sup> In our recent study, we found a strong association between AFB<sub>1</sub> exposure and liver cirrhosis.<sup>9</sup> AFB<sub>1</sub> is mainly metabolized by CYP3A4 into the reactive free

radical AFB<sub>1</sub>-exo-8, 9-epoxide.<sup>10</sup> This intermediate metabolite is typically detoxified by conjugation with glutathione through the action of GST enzymes, mainly GSTM1 and GSTT1 encoded by the *GSTM1* and *GSTT1* gene, respectively.<sup>11</sup> Copy number variation (CNV) has been ascribed to these genes and individuals with zero copy number (null genotype) of *GSTM1* and/or *GSTT1* are more prone to develop HCC associated with AFB<sub>1</sub> exposure.<sup>12,13</sup> However, previous reports failed to distinguish between heterozygous and homozygous bearers of the non-deleted alleles. Hence, the present study aimed at investigating *GSTT1* and *GSTM1* CNVs and the risk for liver cirrhosis in Eastern Ethiopia.

## Materials and Methods

### Study Subjects

This is a follow-up of a case–control study conducted from January 2020 – July 2021 to assess the risk factors associated with liver cirrhosis in the Internal Medicine Unit of Hiwot Fana Comprehensive Specialized University Hospital (HFCSUH), Harar, Eastern Ethiopia.<sup>9</sup> In brief, the study consisted of 127 confirmed cases of liver cirrhosis and 253 controls without any history or clinical evidence of liver diseases. Written consent from the participants was obtained after explaining aims of the study and a detailed questionnaire was filled out for each case and control by a trained interviewer. A subset of 210 individuals, including all cases (106), for whom blood samples were available, and an equivalent number of controls (104) were randomly selected and included in this genetic association study.

### Blood Collection

Two mL of blood was collected in an EDTA tube for genomic DNA extraction. Additional 3–5 mL blood was also taken in a serum separator tube for clinical chemistry tests. Whole blood samples collected for genomic DNA extraction were stored at –80°C until transport and analysis at the Armauer Hansen Research Institute, Addis Ababa, Ethiopia.

### DNA Extraction

Genomic DNA was isolated from whole blood using PureLink™ Genomic DNA Mini Kit (Invitrogen) according to the manufacturer's instructions. DNA quality and quantity was assessed using NanoDrop 2000 spectrophotometer (ThermoFisher Scientific, USA). Moreover, 10% of the samples were randomly selected and agarose gel electrophoresis with the aid of a gel imaging system, Gel Doc (Bio-Rad), was performed to ensure quality of the extracted DNA. The genomic DNA samples were stored at –20°C until analysis.

### *GSTM1* and *GSTT1* Copy Number Analysis

Copy number determination was carried out by a Bio-Rad CFX96 Touch Real-Time System. The reaction mixture (10 uL) was composed of FAM-labeled 20x TaqMan™ Copy Number Assay kit (AB Applied Biosystems) for *GSTM1* (Hs02595872\_cn) or *GSTT1* (Hs01731033\_cn), VIC-labeled 20X TaqMan™ Reference Assay kit (AB Applied Biosystems: 4403326), TaqPath™ ProAmp™ Master Mix (Applied Biosystems), nuclease-free water and 10 ng genomic DNA template. Thermocycling was initiated at 95°C for 10 min followed by 40 cycles of 15 sec of denaturation at 95°C and 60-sec annealing-extension at 60°C.

To ensure quality, each target assay was done in the same PCR as RNaseP. The amplification of RNaseP and GST genes in the same sample adjusted DNA concentration differences between samples and ensured that no false-negative GST\*0/0 genotypes were created due to PCR or pipetting failure or inadequate DNA concentrations in the original sample. The genotyping was done blind to the subject's case or control status. The samples were run twice. In addition, each run contained a no-template control to rule out any contamination. The number of amplification cycles necessary for the fluorescent signal to reach the threshold and surpass the background level is known as the cycle threshold (Ct) value in the qPCR experiment. All samples were quantified in duplicates and average Ct values were normalized to *RNase P* (the reference gene with copy number 2). Copy number estimation was conducted by the  $\Delta\Delta C_t$  method as follows:

$$\text{Ct target gene} = 2 \times 2^{-\Delta\Delta\text{Ct}}$$

where  $\Delta\Delta\text{Ct} = \Delta\text{Ct}_{\text{GST sample}} - \Delta\text{Ct}_{\text{RNase P}}$  and  $\Delta\text{Ct}$  was the average Ct value of 2 repeated measurements of the sample. The result obtained from the  $2^{-\Delta\Delta\text{Ct}}$  calculation was multiplied by 2 because the target genes are known to be diploid.<sup>14,15</sup>

## Clinical Chemistry

Liver function test (LFT) including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), serum bilirubin (total), albumin, and serum creatinine was determined using Cobas C-311 fully automated closed clinical chemistry analyzer (Roche/Hitachi Cobas C-311, Roche diagnostics GmbH, Mannheim, Germany).

## Statistical Analysis

Categorical variables were expressed as frequencies and percentages, while continuous variables were expressed as median (IQR). The difference between cases and controls in terms of categorical and continuous variables was tested using  $\chi^2$  or Fisher's exact test and the nonparametric Mann-Whitney-*U* test, respectively. Using unconditional logistic regression, odds ratios (ORs) were estimated for *GSTM1* and *GSTT1* genotypes, comparing groups defined by the gene copy number coded as a categorical variable (0, 1, or  $\geq 2$  copies), null, and carriers (1, 2 or more copies). Given that gender and age are biological risk factors for liver cirrhosis, we adjusted the OR for these variables as potential confounders. The association between clinical chemistry tests and *GSTM1* and *GSTT1* copy numbers and genotypes was also investigated using linear regression. Since the Shapiro-Wilk test revealed a non-normal distribution of the clinical chemistry data, we used the log-transformed data during regression analysis. The adjustment was made using the potential confounders (age and gender).  $P < 0.05$  was considered statistically significant. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 26.0.

## Ethical Considerations

This study was conducted following the Declaration of Helsinki. Ethical approval was obtained from both the Institutional Review Board of the College of Health Sciences, Addis Ababa University (Protocol No. 064/19/SOP and Reference No. CHS/RTTD/229/2020) and the National Ethical Review Committee of the Ministry of Science and Higher Education (Reference No. 04/246/680/21). Written consent was obtained following provision of information regarding the study's objectives, benefits, and risks to participants. Confidentiality and anonymity were ensured by restricting data access and removing identifiers, respectively.

## Results

### Sociodemographic and Clinical Characteristics

Table 1 shows characteristics of the study participants. Males accounted for a higher number of cases (67%) than controls (52%) and we found statistically significant differences ( $p < 0.05$ ) regarding gender but not age between the two groups. There was a significant ( $p < 0.05$ ) difference between cases and controls in terms of all clinical chemistry parameters (Table 1).

### Copy Number Variation

Figure 1 depicts CNV of the study participants. The *GSTM1* null genotype was more common in cases (0.45; 95% CI: 0.36, 0.55) than controls (0.33; 95% CI: 0.24, 0.43), with an overall frequency of 0.39 (95% CI: 0.32, 0.46) in the study population. Similarly, the frequency of *GSTT1* null genotype was significantly higher ( $p < 0.05$ ) in cases (0.40; 95% CI: 0.30, 0.50) than controls (0.25; 95% CI: 0.17, 0.34), with an overall frequency of 0.32 (95% CI: 0.26, 0.39). The overall percentage of double null *GSTM1* and *GSTT1* genotypes was 13% (95% CI: 0.09, 0.19) and relatively more common in cases (16%; 95% CI: 0.10, 0.24) than controls (11%; 95% CI: 0.05, 0.18). Moreover, 3.3% (95% CI: 0.01, 0.07) of the study participants had  $> 2$  copies of the *GSTM1* or *GSTT1* genes, each accounting for 1.9% (95% CI: 0.01, 0.05) and 1.4% (95% CI: 0.01, 0.04) for *GSTT1*, respectively.

**Table 1** Sociodemographic and Clinical Chemistry Characteristics of the Study Participants, Eastern Ethiopia, 2020/21 (N=210)

Characteristics	Cases n (%)	Controls n (%)
Gender*		
Male	71 (67)	54 (52)
Female	35 (33)	50 (48)
Age	32 (28–50)	34 (26–50)
<35	54 (51)	52 (50)
35–44	17 (16)	22 (21)
45–54	16 (15)	8 (8)
>55	19 (18)	22 (21)
ALT (U/L)*	40 (19.8–53.3)	19 (14.7–26)
AST (U/L)*	52.3 (32–76.7)	21 (16.7–28)
ALP (U/L)*	164 (94–261.3)	86 (67–111)
Bilirubin, total (μmol/L)*	18.7 (8.5–32.7)	6.8 (3.8–14.7)
Albumin (g/L)*	29 (22.8–38.3)	41 (33.3–45)
Creatinine (μmol/L)*	63.5 (48.8–81.8)	55 (45.3–65)

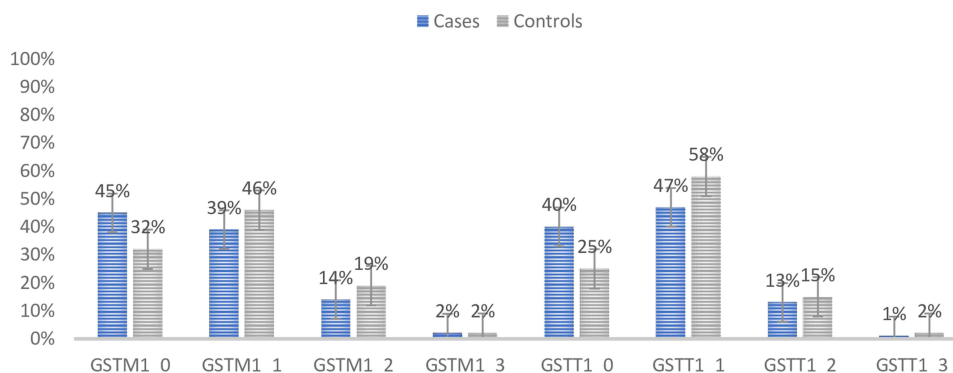
**Notes:** N=210; n for cases=106; n for controls=104; data are presented as number (%) or as median (interquartile range); \*p-value <0.05 from the chi-square test or Fisher exact test for categorical and Mann–Whitney U-test for continuous variables.

**Abbreviations:** ALT, alanine aminotransferase; AST, Aspartate aminotransferase; ALP, alkaline phosphatase; g/L, gram per liter; μmol/L, micromole per liter; U/L, units per liter.

## Association Studies

As shown in Table 2, *GSTT1* carriers had a significantly ( $p<0.05$ ) lower risk of liver cirrhosis than individuals with null genotypes (AOR=0.47; 95% CI: 0.25, 0.86). Further analysis also revealed that patients with one copy of the *GSTT1* gene had a significantly ( $p<0.05$ ) lower risk of liver cirrhosis than patients with null genotypes (AOR=0.48; 95% CI: 0.25, 0.91). Moreover, the risk of liver cirrhosis was markedly ( $p<0.05$ ) decreased in those patients who had  $\geq 2$  combined *GSTM1* and *GSTT1* copies compared with patients who had a dual null genotype (AOR=0.38; 95% CI: 0.16, 0.91). There was also a significant gene dose relationship ( $p$ -trend < 0.001).

We further investigated whether *GSTM1* and *GSTT1* copy numbers and genotypes indirectly contribute to the levels of clinical chemistry parameters as shown in Table 3. Consequently, the ALT level was reduced by 31% ( $\beta = 0.69$ ; 95% CI: 0.48, 0.99;  $p<0.05$ ) in cases with  $\geq 2$  copies of *GSTM1* in comparison with null genotypes (Table 3). Moreover, AST level was decreased by 39% ( $\beta = 0.61$ ; 95% CI: 0.41, 0.90;  $p<0.05$ ) in cases having  $\geq 2$  copies of *GSTT1* than those with

**Figure 1** Frequency of *GSTM1* and *GSTT1* copy numbers among the study participants, Eastern Ethiopia, 2020/21 (n=210).

**Table 2** Risk of Liver Cirrhosis in Relation to *GSTM1* and *GSTT1* Genes Copy Numbers and Genotypes, Eastern Ethiopia, 2020/21 (N=210)

Copy Numbers and Genotype	Cases (%)	Controls n (%)	Crude OR	Adjusted OR <sup>a</sup>
<i>GSTM1</i>				
0	48 (45)	34 (33)	1.00	1.00
1	41 (39)	48 (46)	0.61 (0.33, 1.11)	0.57 (0.31, 1.07)
≥2	17 (16)	22 (21)	0.55 (0.25, 1.18)	0.56 (0.25, 1.25)
<i>p</i> trend			0.17	0.16
Null	48 (45)	34 (32)	1.00	1.00
Carrier	58 (55)	70 (67)	0.62 (0.34, 1.03)	0.57 (0.32, 1.02)
<i>GSTT1</i>				
0	42 (40)	26 (25)	1.00	1.00
1	49 (46)	60 (58)	0.51 (0.27, 0.94)	0.48 (0.25, 0.91) *
≥2	15 (14)	18 (17)	0.52 (0.22, 1.20)	0.43 (0.18, 1.04)
<i>p</i> trend			0.08	0.05
Null	42 (40)	26 (25)	1.00	1.00
Carrier	64 (60)	78 (75)	0.51 (0.28, 0.92)	0.47 (0.25, 0.86) *
Sum of <i>GSTM1</i> and <i>GSTT1</i> <sup>b</sup>				
0	17 (16)	11 (11)	1.00	1.00
1	42 (40)	25 (24)	1.09 (0.44, 2.69)	0.92 (0.37, 2.43)
≥2	47 (44)	68 (65)	0.45 (0.19, 1.04)	0.38 (0.16, 0.91) *
<i>p</i> trend			0.01	<0.001
Null/Null	17 (16)	11 (11)	1.00	1.00
Carrier/Carrier	89 (84)	93 (89)	0.62 (0.28, 1.40)	0.54 (0.23, 1.25)

**Notes:** N=210; n for cases=106; n for controls=104; \**p*-value <0.05; <sup>a</sup>Odds ratio adjusted for gender and age; <sup>b</sup>0=*GSTM1*<sub>0</sub> + *GSTT1*<sub>0</sub>, 1=*GSTM1*<sub>0</sub> + *GSTT1*<sub>1</sub> or *GSTM1*<sub>1</sub> + *GSTT1*<sub>0</sub>, 2=*GSTM1*<sub>1</sub> + *GSTT1*<sub>1</sub> or *GSTM1*<sub>0</sub> + *GSTT1*<sub>2</sub> or *GSTM1*<sub>2</sub> + *GSTT1*<sub>0</sub>, ≥3=*GSTM1*<sub>0</sub> + *GSTT1*<sub>3</sub> or *GSTM1*<sub>3</sub> + *GSTT1*<sub>0</sub> or *GSTM1*<sub>2</sub> + *GSTT1*<sub>2</sub> or *GSTM1*<sub>1</sub> + *GSTT1*<sub>2</sub> or *GSTM1*<sub>2</sub> + *GSTT1*<sub>1</sub> or *GSTM1*<sub>1</sub> + *GSTT1*<sub>3</sub> or *GSTM1*<sub>3</sub> + *GSTT1*<sub>1</sub>.  
**Abbreviations:** *GSTM1*, glutathione S-transferase M; *GSTT1*, glutathione S-transferase T1; OR, odds ratio.

**Table 3** Linear Regression of the Association Between ALT, AST, *GSTM1*, and *GSTT1* Copy Numbers and Genotypes, Eastern Ethiopia, 2020/21 (N=210)

Copy Numbers and Genotype	ALT (U/L)		AST (U/L)	
	Cases β (95% CI) <sup>a</sup>	Controls β (95% CI) <sup>a</sup>	Cases β (95% CI) <sup>a</sup>	Controls β (95% CI) <sup>a</sup>
<i>GSTM1</i>				
0	1.00	1.00	1.00	1.00
1	1.18 (0.90, 1.55)	1.09 (0.89, 1.34)	1.25 (0.95, 1.63)	0.99 (0.82, 1.20)
≥2	0.69 (0.48, 0.99)*	1.15 (0.90, 1.48)	0.70 (0.49, 1.01)	0.90 (0.70, 1.13)
Null	1.00	1.00	1.00	1.00
Carrier	1.01 (0.79, 1.31)	1.11 (0.91, 1.33)	1.05 (0.81, 1.36)	0.96 (0.80, 1.14)
<i>GSTT1</i>				
0	1.00	1.00	1.00	1.00
1	0.89 (0.64, 1.16)	0.89 (0.72, 1.09)	0.81 (0.62, 1.06)	0.93 (0.76, 1.13)
≥2	1.01 (0.68, 1.51)	0.68 (0.52, 1.12)	0.61 (0.41, 0.90)*	0.79 (0.62, 1.03)
Null	1.00	1.00	1.00	1.00
Carrier	0.91 (0.70, 1.19)	0.84 (0.68, 1.02)	0.76 (0.58, 0.98)*	0.90 (0.74, 1.08)
Sum of <i>GSTM1</i> and <i>GSTT1</i> <sup>b</sup>				
0	1.00	1.00	1.00	1.00
1	0.83 (0.57, 1.21)	0.79 (0.57, 1.11)	1.19 (0.82, 1.72)	0.86 (0.63, 1.16)

(Continued)

**Table 3** (Continued).

Copy Numbers and Genotype	ALT (U/L)		AST (U/L)	
	Cases $\beta$ (95% CI) <sup>a</sup>	Controls $\beta$ (95% CI) <sup>a</sup>	Cases $\beta$ (95% CI) <sup>a</sup>	Controls $\beta$ (95% CI) <sup>a</sup>
$\geq 2$	0.83 (0.57, 1.21)	0.86 (0.64, 1.16)	0.82 (0.57, 1.17)	0.84 (0.64, 1.09)
Null/Null	1.00	1.00	1.00	
Carrier/Carrier	0.83 (0.59, 1.17)	0.84 (0.63, 1.13)	0.98 (0.68, 1.39)	0.84 (0.64, 1.09)

**Notes:** N= 210; n for cases=106; n for controls=104; \*p-value <0.05; <sup>a</sup>Adjusted for age and gender; <sup>b</sup>0=*GSTM1*<sub>0</sub> + *GSTT1*<sub>0</sub>, 1=*GSTM1*<sub>0</sub> + *GSTT1*<sub>1</sub> or *GSTM1*<sub>1</sub> + *GSTT1*<sub>0</sub>, 2= *GSTM1*<sub>1</sub> + *GSTT1*<sub>1</sub> or *GSTM1*<sub>0</sub> + *GSTT1*<sub>2</sub> or *GSTM1*<sub>2</sub> + *GSTT1*<sub>0</sub>,  $\geq 3$ = *GSTM1*<sub>0</sub> + *GSTT1*<sub>3</sub> or *GSTM1*<sub>3</sub> + *GSTT1*<sub>0</sub> or *GSTM1*<sub>2</sub> + *GSTT1*<sub>2</sub> or *GSTM1*<sub>1</sub> + *GSTT1*<sub>2</sub> or *GSTM1*<sub>2</sub> + *GSTT1*<sub>1</sub> or *GSTM1*<sub>1</sub> + *GSTT1*<sub>3</sub> or *GSTM1*<sub>3</sub> + *GSTT1*<sub>1</sub>.

**Abbreviations:** ALT, alanine aminotransferase; AST, Aspartate aminotransferase. *GSTM1*, glutathione S-transferase M; *GSTT1*, glutathione S-transferase T1; U/L, units per liter.

a null genotype. Similarly, AST level showed a significant ( $p < 0.05$ ) reduction in cases who were carriers of the *GSTT1* genotype compared to null genotypes ( $\beta = 0.76$ ; 95% CI: 0.58, 0.98) (Table 3).

## Discussion

Previous studies suggest that polymorphisms in genes encoding Phase II metabolizing enzymes, such as *GSTM1* and *GSTT1* may be linked to an increased vulnerability to various disorders associated with oxidative stress.<sup>16,17</sup> To the best of our knowledge, this is the first study showing the contribution of the exact CNV of *GSTM1* and *GSTT1* to liver cirrhosis. In our study, both *GSTM1* and *GSTT1* null genotypes were more frequently detected in liver cirrhosis patients than controls and the overall frequency was 0.39 and 0.32, respectively. Furthermore, 13% of the study population had double *GSTM1* and *GSTT1* null genotypes, whereas 3.3% (1.9% for *GSTM1* and 1.4% for *GSTT1*) had more than two copies of the *GSTM1* or *GSTT1* genes.

The frequency of *GSTM1* and *GSTT1* null genotypes varies in different populations. Consequently, *GSTM1* null genotype was more frequent in Asians (0.23 to 0.66),<sup>16,18</sup> Caucasians (0.45 to 0.57),<sup>16,19</sup> and Arabs (0.50 to 0.63)<sup>20,21</sup> compared to Africans (0.11 to 0.44).<sup>19</sup> On the other hand, *GSTT1* null genotype was shown to be more frequent among Asians (0.35 to 0.52),<sup>16,18</sup> Africans (0.20 to 0.47)<sup>19</sup> and Arabs (0.20 to 0.37)<sup>20,21</sup> in contrast to Caucasians (0.14 to 0.28).<sup>16,19</sup> The highest frequency of double *GSTM1* and *GSTT1* null genotypes was observed in Asians (0.27),<sup>22</sup> followed by Arabs (0.21),<sup>21</sup> and Africans (0.15).<sup>23</sup> Furthermore, evidence for *GSTM1* or *GSTT1* gene duplication has been identified at a significantly higher frequency in a study involving a Caribbean population descended from Africa (3.4%) than Caucasians (0.2%).<sup>24,25</sup> Indeed, our findings on the frequency of individual and combined (double) null genotypes and the percentage of more than two copies of the genes are consistent with previous studies involving Africans and populations descended from Africa.

In our study, *GSTT1* carriers had a significantly lower risk of liver cirrhosis than individuals with null genotypes, and specifically, patients with one copy of the *GSTT1* gene had a significantly lower risk of liver cirrhosis compared with null genotypes. Furthermore, patients with  $\geq 2$  combined *GSTM1* and *GSTT1* copies had a significantly lower risk of liver cirrhosis than those with a dual null genotype. In this regard, no observational studies exploring the relation between *GSTT1* and *GSTM1* copy numbers and the risk of liver cirrhosis are available for comparison. However, several studies have reported the association between *GSTM1* and *GSTT1* polymorphisms and the risk of liver disease, including drug-induced liver injury,<sup>6,26</sup> alcoholic cirrhosis,<sup>27</sup> viral and non-viral HCC,<sup>28</sup> non-alcoholic fatty liver disease,<sup>29</sup> and HCV associated cirrhosis.<sup>30</sup> However, all previous studies reported the association based on comparing carriers (irrespective of copy number of the genes) and null genotypes of *GSTM1* and *GSTT1*. For instance, a recent meta-analysis reported an increased risk of HCC in patients with *GSTM1* or *GSTT1* null genotypes, and double null genotypes of *GSTM1* and *GSTT1*.<sup>28</sup>

Our study also demonstrated a significant gene dose relationship between the sum of *GSTM1* and *GSTT1* copies and the risk of liver cirrhosis. This could support the notion that the activity of enzymes encoded by *GSTM1* and *GSTT1* genes is directly proportional to their copy numbers.<sup>31,32</sup> Thus, having more copy numbers could be protective against

oxidative stress and the associated inflammation due to various insults to the hepatocytes, including viral and non-viral agents.

We further investigated whether *GSTM1* and *GSTT1* genotypes indirectly contribute to the levels of clinical chemistry tests. Accordingly,  $\geq 2$  copies of combined *GSTM1* and *GSTT1* genes were associated with a significant reduction in ALT and AST levels, respectively. Moreover, a significant decrease in AST level was observed in *GSTT1* carriers compared with null genotypes. To the best of our knowledge, there are no studies focusing on the impact of *GSTM1* and *GSTT1* CNVs on clinical chemistry parameters of liver cirrhosis patients. There are, however, few studies that attempted to investigate the contribution of *GSTM1* and *GSTT1* to the liver biochemistry tests. For example, a study conducted among HCV-infected Brazilian cirrhosis and HCC patients reported no significant difference between carriers and null genotypes of these genes in terms of the blood level of clinical chemistry parameters.<sup>30</sup> By contrast, our study demonstrated significant difference in AST levels between *GSTT1* carriers and null genotypes of liver cirrhosis patients apart from the observed differences in  $\geq 2$  copies of combined *GSTM1* and *GSTT1* mentioned above. This discrepancy might arise from the fact that the mentioned study compared only null vs carriers of the genes but our study further investigated the copy numbers in carriers of the genes. Besides, differences in race and etiology of liver cirrhosis could also explain the discrepancies. Exposure to HBV and AFB<sub>1</sub> are the most important etiologies of HCC and liver cirrhosis in Africa and Asia, whereas HCV infection and alcohol consumption are the main drivers in America, Oceania, and Europe.<sup>33–35</sup> Indeed, in our recent study, HBV infection and AFB<sub>1</sub> exposure were the major culprits of liver cirrhosis in Eastern Ethiopia.<sup>9</sup>

On the other hand, few studies have reported the significance of null genotypes of the *GSTM1* and *GSTT1* genes for changes in the serum levels of ALT and AST due to exposure to hepatotoxic drugs, which have reactive free radicals as intermediate metabolites.<sup>6,36,37</sup> For instance, a study done among Tunisian epileptic patients treated with carbamazepine, known to have 10, 11-epoxy carbamazepine as a reactive metabolite, reported the association between *GSTM1* null genotype and elevation in ALT and AST, whereas AST elevation was associated with *GSTT1* null genotype.<sup>6</sup> Thus, functional *GSTM1* and *GSTT1* genes might indirectly contribute to the level of AST and ALT, at least by minimizing oxidative stress and associated damage due to reactive oxygen species produced as a result of drugs and environmental toxins, including AFB<sub>1</sub> in the hepatocytes. However, additional studies with larger sample size and inclusion of controls with a risk factor would be required for substantiating these observations.

## Conclusions

Our findings suggest that a single copy number of *GSTT1*, and  $\geq 2$  copies of combined *GSTM1* and *GSTT1* are associated with reduced risk of liver cirrhosis in Ethiopians. Besides,  $\geq 2$  copies of *GSTM1* and *GSTT1* genes were associated with a substantial decrease in ALT and AST levels, respectively. These findings highlight the significance of gene–environment interactions in the multifactorial development of liver cirrhosis.

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## Author Contributions

All authors made a significant contribution to the work reported whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Disclosure

The authors declare that there are no conflicts of interest in this work.

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