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The role of *GSTM1* and *GSTT1* deletion variants and alcohol consumption profile in the development of alcoholic liver cirrhosis

Улога делеционих варијанти *ГСТМ1* и *ГСТТ1* и профила конзумирања алкохола у развоју алкохолне цирозе јетре

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SUMMARY

Introduction/Objective Alcoholic liver cirrhosis (ALC) is the primary cause of alcohol abuse-related mortality, resulting from alcohol-induced oxidative stress. Glutathione S-transferases (GSTM1, GSTT1) are enzymes essential for detoxification, and their deficiency may contribute to the onset of chronic inflammation and disease progression. This study sought to investigate the relationship between *GSTM1* and *GSTT1* deletions and ALC, as well as alcohol consumption patterns in cirrhosis development.

Methods The analysis included 114 ALC patients and 262 controls, with *GSTM1* and *GSTT1* deletions assessed via multiplex PCR.

Results Findings indicated that individuals with the *GSTM1* null genotype had a three-fold increased risk of developing ALC (95% CI, 1.87–4.81; p < 0.0001), whereas *GSTT1* null genotypes showed no significant impact. Individuals with both *GSTM1* null and *GSTT1* null genotypes exhibited an 11-fold heightened risk of ALC (OR = 11.21, 95% CI = 3.30–38.14, p < 0.001). Furthermore, patients who commenced alcohol consumption at 22.5 years or older developed cirrhosis more rapidly than their younger counterparts (p < 0.001).

Conclusion *GSTM1* null and combined *GSTM1/GSTT1* null genotypes constitute significant risk factors for ALC, with older patients experiencing accelerated disease progression irrespective of alcohol intake levels.

Keywords: alcoholic liver cirrhosis (ALC); drinking profile; *GSTM1* and *GSTT1*; deletion variants; null genotypes

САЖЕТАК

Увод/Циљ Алкохолна цироза јетре (АЦЈ) је примарни узрок смртности узроковане злоупотребом алкохола, насталог као последица алкохолом изазваног оксидативног стреса. Глутатион *S*-трансферазе (ГСТМ1, ГСТТ1) јесу ензими неопходни за детоксификацију и њихов недостатак може утицати на појаву хроничне инфламације и прогресију болести. Ова студија је имала за циљ да истражи повезаност између *ГСТМ1* и *ГСТТ1* делеционих варианти и АЦЈ, као и профила пијења алкохола, у развоју цирозе.

Методе Анализа је обухватила 114 пацијената са АЦЈ и 262 контроле, а мултиплекс ПЦР-ом су одређене делеционе варијанте *ГСТМ1* и *ГСТТ1*.

Резултати Резултати су показали да особе са *ГСТМ1* нултим генотипом имају три пута повећан ризик од развоја АЦЈ (95% *CI*, 1,87–4,81; $p < 0,0001$), при чему *ГСТТ1* нулти генотипови нису показали значајан утицај на развој болести. Особе са оба нулта генотипа *ГСТМ1/ГСТТ1* показале су 11 пута повећан ризик од АЦЈ (*OR* = 11,21, 95% *CI* = 3,30–38,14; $p < 0,001$). Пацијенти који су почели да конзумирају алкохол са 22,5 или више година брже су развили цирозу од оних који су почели да пију у млађем узрасту ($p < 0,001$).

Закључак *ГСТМ1* нулти и *ГСТМ1/ГСТТ1* комбиновани нулти генотипови представљају значајне факторе ризика за развој АЦЈ код старијих пацијената код којих је убрзана прогресија болести без обзира на ниво уноса алкохола.

Кључне речи: алкохолна цироза јетре (АЦЈ); профил пијења алкохола; *ГСТМ1* и *ГСТТ1*; делеционе варијанте; нулти генотипови

INTRODUCTION

The consumption of alcoholic beverages causes approximately three million global deaths (5.3%) annually, mostly due to liver failure. Fatalities linked to alcoholic liver disease (ALD) constitute ~ 21.3%, establishing ALD as one of the primary causes of alcohol-related mortality [1, 2]. ALD encompasses a spectrum of hepatic disorders (alcohol-associated steatosis, steatohepatitis, cirrhosis, and hepatocellular carcinoma) associated with prolonged alcohol intake [3].

Alcoholic liver cirrhosis (ALC) is a multifactorial disease influenced by environmental, behavioral, metabolic, and genetic factors. It develops following an extended period of chronic liver inflammation, usually caused by long-term alcohol intake. The risk of ALC correlates with

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drinking patterns, and its progression increases significantly with consumption exceeding three drinks/day for men and two for women [4]. However, chronic liver inflammation does not always progress to cirrhosis, and the effects of alcohol consumption vary among individuals with equivalent intake levels [5, 6].

Phase II metabolizing enzymes, such as glutathione S-transferases (GSTs), protect cells from oxidative stress, particuralry from secondary cytotoxic metabolites of reactive oxidative species (ROS) [7]. Homozygous deletions of cytosolic GST θ and μ enzymes, encoded by *GSTT1* and *GSTM1* genes ("null" genotypes), are associated with the absence of these enzymes, increasing susceptibility to ROS, and predisposing hepatocytes to chronic liver inflammation, tissue damage, and ALC development.

Given the inconsistent findings regarding the association between *GSTM1* and *GSTT1* deletion variants and ALC [8–11], we investigated the association between *GSTM1* and *GSTT1* gene deletion variants and alcohol consumption patterns and susceptibility to ALC onset.

METHODS

Study participants

114 patients (12 females and 102 males), diagnosed with ALC at the Clinic of Gastroenterology and Hepatology, University Hospital Medical Center "Zvezdara", Belgrade, Serbia, between 2015 and 2018, were included. ALC is diagnosed in the presence of clinical or biological signs of liver damage, in individuals consuming more than 20g/day in women (≈2 drinks) or 30g/day in men (≈3 drinks) [4].

Liver cirrhosis was diagnosed under standard clinical and laboratory criteria. Comprehensive blood analyses, including complete blood counts, electrolytes, and biochemical markers for the diagnosis of ALC in liver functional tests (LFT): alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT), coagulation status (prothrombin time-PT/international normalized ratio-INR), serum albumin, and serum bilirubin concentrations were conducted. Radiological imaging confirmed signs of cirrhosis. Ultrasonography (US) and computed tomography (CT) verified the presence of a nodular liver surface, splenomegaly, collateral vessels, and ascites, while esophagogastroduodenoscopy (EGD) was conducted to screen for esophageal varices. Neuropsychological testing was performed to detect hepatic encephalopathy (confusion, asterixis, fetor hepaticus). In several patients, the diagnosis was established through liver biopsy.

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The severity of liver failure was assessed utilizing the LFT through the Child-Pugh (CP) scoring system. Three CP categories were present: A (asymptomatic or compensated cirrhosis, low mortality risk), B (intermediate disease with moderately impaired hepatic function, decompensated cirrhosis), and C (decompensated cirrhosis, the most severe form with advanced hepatic dysfunction) [6].

The drinking profile, namely the volume of daily alcohol consumption, duration of regular drinking, age at which drinking started, and the type of beverage (beer, wine, or spirits), was quantitatively recorded at the hospital's first visit. Daily alcohol intake (g) was calculated as the number of standard drinks ×10 g. One drink was 100ml of wine (13%), 30ml of spirits (40%), or 250ml of beer (5%). Alcohol exposure duration was estimated from the self-reported drinking onset age to cirrhosis diagnosis.

The control group comprised 262 subjects who came for a preventive health check to Zvezdara University Hospital Medical Center or were blood donors, who self-reported as either abstainers or individuals who consumed < 10 g of alcohol/day with no evidence of liver disease or other pathological conditions.

All participants were unrelated and of Serbian origin. Written informed consent was obtained from all study participants. This study was conducted in accordance with the Declaration of Helsinki, and the Ethics Committee of the Zvezdara University Hospital Medical Center approved the study (Approval Reg. No 8-6-2018, from 06-01-2018).

Genotyping

Blood samples of study participants were collected in EDTA-coated vacutainers. Genomic DNA was extracted using a DNA extraction kit (Gene JET Whole Blood Genomic DNA Purification Mini Kit; Thermo Scientific, USA) according to the manufacturer's protocol. DNA was stored at -20°C until further analysis.

The genotyping was done by multiplex polymerase chain reaction (multiplex PCR) for *GSTM1* and *GSTT1*, with the *β-globin* gene as an internal control. Multiplex PCR reactions contained 2X Multiplex PCR Master Mix (Qiagen®, Hilden, Germany), 0.5μM of each primer (Metabion, Planegg, Germany) and 0.2μg of genomic DNA [12]. PCR products were separated on 3% agarose gel, stained with GreenSafe (NZYtech, Lisboa, Portugal), and visualized under UV light. *GSTM1* and *GSTT1* null genotypes displayed no fragments corresponding to 215bp for *GSTM1* and 480bp for *GSTT1*, and the 110bp for the control *β-globin* gene was observed in every PCR reaction. For validation, 10% of samples were randomly selected and re-genotyped.

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Statistical analysis

The chi-squared test (χ^2) was used to evaluate differences between groups for categorical variables. Normally distributed continuous variables were analyzed using the independent t-test and one-way ANOVA, as appropriate, while non-normally distributed variables were analyzed with the Mann–Whitney or Kruskal–Wallis test. Genotype frequencies were directly counted. Univariate binary logistic regression assessed the association between different genotypes and the development of ALC. To determine the relationship between the drinking profile and the duration of alcohol exposure prior to the diagnosis of cirrhosis, Pearson correlation coefficients were calculated. Kaplan-Meier curves analyzed the time to decompensation for patients stratified by median age at the start of drinking alcoholic beverages, and compared statistically using the log-rank test. The commencement of the curves was the self-reported time at the initiation of alcohol consumption, and the follow-up time was the duration of alcohol exposure prior to the diagnosis of cirrhosis. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS version 20.0, SPSS Inc., Chicago, USA) with statistical significance at $P < 0.05$.

To identify patients at elevated risk of developing ALC, polygenic risk scores (PRSs) based on two deletion variants were developed. It was calculated using R v.4.3.0, as a function $f_\beta(x) = \frac{\sum_{i=1}^{n} \beta_i g(x_i)}{\sum_{i=1}^{n} \beta i}$, where x_i represents points assigned to subjects based on deletion status. Homozygous carriers of *GSTM1* and *GSTT1* deletions were assigned 1 point for each gene; heterozygous carriers, 0.5; and carriers of wild-type alleles, 0. The effect weights of deletions, β, were calculated from a GWA study [10]. For *GSTM1* (null genotype/deletion), β was 1.308333, and for *GSTT1* (null genotype/deletion), β was 0.69. Differences in PRS distribution between controls and ALC patients were assessed using the nonparametric Wilcoxon rank-sum test for continuous data, with statistical significance at $P < 0.05$.

Ethics: The study was approved by the Ethics Committee of the Zvezdara University Hospital Medical Center (Approval Reg.No 8-6-2018, from 06-01-2018).

RESULTS

Study participants

Nine times fewer females than males were observed (12 vs. 102 in the ALC group and 30 vs. 232 in the control group, respectively, $P = 0.736$). The mean age of participants in the ALC and control groups was 58.23 and 58.56 years, respectively ($P = 0.794$). No statistically

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significant differences were observed between patients with ALC and control subjects regarding age or sex distribution.

At the time of hospital admission, patients in CP class A, exhibiting asymptomatic and compensated cirrhosis, were the least represented (14 patients, 12.3%). In contrast, patients with decompensated cirrhosis, both with advanced disease (CP class C, 53 patients, 46.5%), and with CP class B (47 cases, 41.2%) were more prevalent. The median age of onset for alcohol consumption among the patients with ALC was 22.5 years (19-30 years). On average, alcohol consumption commenced 34.6 years prior to cirrhosis diagnosis, with a median daily alcohol intake of 72g (60-91.5g). The majority of patients with ALC consumed spirits (76.3%), followed by beer (71%), and 21.1% reported wine consumption. As participants commonly consumed more than one type of beverage, these categories were not mutually exclusive.

Association between *GSTM1* and *GSTT1* deletion variants and onset of ALC

The *GSTM1* and *GSTT1* genotype frequencies in ALC patients and control subjects are shown in Table 1. The majority of ALC patients had deleted both *GSTM1* gene alleles (null genotype), significantly higher than in control subjects (70.2% vs. 44.3%, $P < 0.001$). The *GSTM1* null genotype was significantly associated with the risk of ALC development ($P < 0.0001$). The risk of developing cirrhosis was three times higher for the *GSTM1* null genotype carriers (OR = 3.00, 95% CI = 1.87-4.81) than in carriers of non-null *GSTM1* genotype. On the contrary, the *GSTT1* null genotype was similarly distributed between patients and control subjects (13.2 vs. 13.0%, $P = 0.96$), with no increased risk of disease onset among *GSTT1* null genotype carriers (OR = 1.02, 95% CI = 0.53-1.96) (Table 1).

Further, patients with ALC were divided into three groups: carriers of *GSTM1* non-null/*GSTT1* non-null genotypes (two active genes), carriers of one deleted gene (*GSTM1* or *GSTT1*; one active gene), and carriers of double null genotypes (*GSTM1* null/*GSTT1* null; no active genes). ALC patients with one active gene had twice the risk of developing ALC compared to those with both active genes (OR = 1.97, 95% CI = 1.22–3.23, $P = 0.007$). Additionally, the significant risk of ALC disease development was associated with ALC patient carriers of combined *GSTTM1* null/*GSTT1* null genotypes (OR = 11.21, 95% CI = 3.30–38.14, $P < 0.001$), showing an eleven-fold higher risk of disease development compared to carriers of *GSTM1* non-null/*GSTT1* non-null genotypes (Table 1).

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Clinical characteristics and drinking profile of ALC patients according to *GSTs* genotypes

Patients with ALC were stratified according to *GST* null genotypes (*GSTM1*: non-null vs. null; *GSTT1*: non-null vs. null; *GSTM1/GSTT1*: null/null vs. 1 active gene and vs. 2 active genes), and drinking profiles, and clinical characteristics (CP class, biochemical laboratory test results) were compared across the groups (Table 2, Table 3). The median age at the onset of at-risk alcohol consumption was similar in all groups (22–23 years). The duration of alcohol exposure was significantly longer among carriers of the *GSTT1* null genotype compared with *GSTT1* non-null carriers (P = 0.006). Patients with an active *GSTM1* gene (non-null genotype) and carriers of two active genes (*GSTM1*/*GSTT1* non-null/non-null genotype) were associated with significantly increased daily alcohol consumption compared to patients without *GSTM1* gene (null genotype) and those with one or two genes deleted (one active gene or null/null genotypes) (P = 0.019 and P = 0.024, respectively). Our results demonstrated that, in terms of beverage type, carriers of the *GSTM1* non-null genotype consumed beer more frequently than carriers of the null genotype (P = 0.029) (Table 2).

The majority of our ALC patients were categorized as CP score class C (45.5%). In this class, patients with *a GSTT1* non-null genotype were more frequent than those with a null genotype (P = 0.049) (Table 3). No significant differences in biochemical laboratory test results were observed among patients with respect to *GSTM1* and *GSTT1* genotypes in the ALC group (Table 2).

No significant correlation was observed between daily alcohol consumption and the duration of alcohol exposure (Pearson correlation, r = 0.036, p = 0.705). However, a significant negative correlation was found between the age at initiation of alcohol consumption and the duration of exposure (r = -0.475, p < 0.001).

Patients 22.5 years of age or older when they initiated drinking developed cirrhosis more rapidly than younger patients (Log-rank P < 0.001) (Figure 1).

2-variant PRS and risk for ALC development

The risk of developing ALC was assessed using PRS, which accounts for deleterious *GSTM1* and *GS*TT1 variants. The distribution of PRSs differed significantly between patients and the control group. The ALC group showed a rightward shift toward higher PRS values compared with control subjects, indicating an increased cumulative genetic risk associated with these deletions. The disparity between the groups was highly significant with p = 2e-05 (Figure 2).

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The *GSTM1* null genotype demonstrated a significant association with ALC, conferring a 3-fold increased risk, whereas the *GSTT1* deletion alone had no effect. The absence of both genes substantially increased the disease risk. Although no genotype-related biochemical differences were detected, the *GSTT1* null genotype was linked to a longer duration of alcohol exposure, and a later onset of drinking correlated with a more rapid progression to cirrhosis. Furthermore, the PRS, incorporating *GSTM1* and *GSTT1* deletion variants, effectively distinguished ALC patients from controls, indicating the contribution of combined genetic and behavioral factors to disease risk.

DISCUSSION

Excessive alcohol consumption causes individual mental, behavioral, medical, and social problems and has a high impact on public health and global economic burden [2, 3, 13].

Our results demonstrated that patients who initiated alcohol consumption later in life progressed to cirrhosis faster than those who started younger. This could reflect a selection bias, as the prevalence of liver disease increases with age [14]. This is likely due to diminished tissue regeneration and impaired metabolism in older individuals [15], making the liver more vulnerable to alcohol-induced injury and fibrosis, ultimately leading to cirrhosis [16]. Some studies have corroborated that a later onset of alcohol consumption is associated with accelerated disease development, which aligns with our findings [17, 18].

Chronic liver inflammation followed by diffuse hepatic fibrosis causes cirrhosis and eventually leads to liver failure [19, 20]. Alcohol metabolism in hepatocytes generates significant ROS, causing cellular injury and lipid peroxidation, increasing oxidative stress and chronic inflammation, which are crucial for the development of ALC [21]. Glutathione S-transferases (GSTs) detoxify harmful electrophilic compounds and ROS by conjugation to reduced glutathione (GSH) [22]. Limited GSH concentrations during stress leave hepatocytes vulnerable to toxic ethanol metabolites [23, 24]. Individuals lacking one or two GST genes exhibit lower antioxidant capacity against elevated ROS levels than those with both active GST genes [25]. In our study, carriers of the *GSTM1* null genotype exhibited a 3-fold increased risk of ALC development, whereas carriers of the double-null genotype (*GSTM1* and *GSTT1*) demonstrated an 11-fold higher risk. This cumulative effect of low GSH concentrations in hepatocytes and the absence of GST enzymes likely contributes to chronic liver inflammation, tissue damage, and cirrhosis. Our results are consistent with the literature data from diverse populations [10, 26]

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and with conditions associated with elevated oxidative stress investigated within the Serbian population [12, 27].

In our study, *GSTT1* null genotype carriers consumed alcohol long before developing ALC (~ 41 years) compared to carriers of at least one *GSTT1* allele (~ 34 years). These findings suggest that the *GSTT1* null genotype did not affect ALC development. A minority of carriers of double-deleted alleles were detected in the group with severe cirrhosis, suggesting that a deficiency in these enzymes may have fatal consequences for patients classified as CP class C. In our patients, carriers of the *GSTM1* null genotype had significantly lower daily alcohol consumption was observed. Furthermore, patients with combined null/null genotypes had significantly lower daily alcohol consumption than patients with one or two active genes. This finding indicates an association between the *GSTM1* null genotype, alone and in combination, and ALC development, irrespective of the quantity of alcohol consumed. Patients with the null/null combination consumed beer at an almost-significantly lower rate and spirits at an almost-significantly higher rate than patients with one or two active genes. These findings may suggest a predisposition to developing cirrhosis irrespective of the type of beverage and daily alcohol intake in individuals possessing double-deleted *GST* genes.

Multiple risk factors and comorbidities can accelerate cirrhosis progression. The combination of null alleles in the *GSTM1* and *GSTT1* genes as a risk factor for developing ALC was further corroborated using PRS calculation. The mean PRS was significantly higher in patients with ALC compared to control subjects. Our findings suggest that PRS may be an effective tool for predicting the risk of developing ALC, as we recently showed in another study [28].

One limitation of this study was the control group selection, comprising individuals without liver or other pathological conditions who self-reported as abstainers or who consumed < 10 g of alcohol/day. This limitation can be mitigated by matching alcohol use disorder (AUD) individuals with patients with similar drinking habits and demographics. Most ALC patients were diagnosed at an advanced stage of liver disease with decompensation; thus, the diagnosis time matched the decompensation time. Assessing daily alcohol intake is challenging because it relies on self-reported data. Patients may be reluctant to report the actual quantities consumed. ALC develops through interactions between genes involved in alcohol metabolism and oxidative stress and environmental factors. Including factors such as body mass index (BMI), diabetes, drinking habits, and additional genetic markers would help estimate the independence of GSTs as cirrhosis risk factors. In addition, no other comorbidities were investigated. The results from this study should be interpreted as those of a single-center study.

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CONCLUSION

Our results demonstrated that *GSTM1* null and combined *GSTM1/GSTT1* null genotypes are significant risk factors for the development of alcoholic liver cirrhosis. In addition, patients who started alcohol consumption at age > 22.5 years develop cirrhosis significantly faster, regardless of the amount of alcohol consumed. Further research on additional genetic variants involved in alcohol metabolism, as well as the examination of other risk factors contributing to the onset and progression of ALC in our patients, should be conducted.

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Data availability: The data underlying this article cannot be shared publicly due to non-disclosure agreement provided by the institution. The data will be shared on the request to the corresponding author.

Conflict of interest: None declared.

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Table 1. *GSTM1* and *GSTT1* genotype frequencies in ALC patients and control subjects

Parameter	Patients N (%) (N = 114)	Control subjects N (%) (N = 262)	Adjusted* odds ratio	Lower 95% CI	Upper 95% CI	p
GSTM1						
Non-null ("+/+","+/-")	34 (29.8)	146 (55.7)	Reference			
Null ("-/-")	80 (70.2)	116 (44.3)	3.00	1.87	4.81	**< 0.001**
GSTT1						
Non-null ("+/+","+/-")	99 (86.8)	228 (87.0)	Reference			
Null ("-/-")	15 (13.2)	34 (13.0)	1.02	0.53	1.96	0.95
GSTs genotypes with active genes						
2 active genes	30 (26.3)	116 (44.3)	Reference			
1 active gene	73 (64.0)	142 (54.2)	1.97	1.22	3.23	**0.007**
No active genes	11(9.7)	4 (1.5)	11.21	3.30	38.14	**< 0.001**

Non-null – at least one copy of the gene; null – deletion of both copies of the gene; active gene – at least one copy of the one gene (*GSTM1* or *GSTT1*); GSTM1 – glutathione S-transferase M1; GSTT1 – glutathione S-transferase T1;

*sex- and age-adjusted, logistic regression

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Table 2. Drinking profile of ALC patients according to *GSTs* genotypes

Drinking profile	GSTM1			GSTT1			GSTM1/GSTT1			
	Non–null N (34)	**Null N (80)**	**p**	**Non–null N (99)**	**Null N (15)**	**p**	**Null/null N (11)**	**One active gene N (73)**	**Two active genes N (30)**	**p**
Initial age of alcohol consumption (years)	22.5 (19–26.5)	22.5 (18–30)	0.78	22 (19–30)	23 (18–26)	0.970	23 (18–30)	22 (18–30)	22.5 (19–26.25)	0.967
Duration of alcohol exposure (years)	35.2 ± 10.5	34.3 ± 9.8	0.63	33.6 ± 9.8	41.1 ± 8.7	**0.006**[a]	40.7 ± 7.8	33.8 ± 10	34.4 ± 10.2	0.093
Daily alcohol consumption (g)	82.5 (60–114)	68.5 (50–83)	**0.019**[b]	72 (60–90)	60 (48–96)	0.664	60 (48–100)	72 (51.5–80)	95 (60–115.5)	**0.024**[c]

The data are expressed as the means ± standard deviations, or medians (25th–75th percentile) unless otherwise noted; active gene – the presence of at least one copy of the one gene (*GSTM1* or *GSTT1*); GSTM1 – glutathione S-transferase M1; GSTT1 – glutathione S-transferase T1;

[a]Student's t-test

[b]Mann–Whitney U test;

[c]Kruskal-Wallis test

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Table 3. Clinical characteristics and drinking profile of alcoholic liver cirrhosis patients according to glutathione S-transferases (*GSTs*) genotypes

Profile/Characteristics		*GSTM1*			*GSTT1*			*GSTM1/GSTT1*			
		Non-null N (34)	**Null N (80)**	**p**	**Non-null N (99)**	**Null N (15)**	**P**	**Null/Null N (11)**	**one active gene N (73)**	**two active genes N (30)**	**p**
Drinking profile											
Type of beverage	beer N (%)	29 (85.3)	52 (65)	0.029[d]	72 (72.7)	9 (60)	0.311	5 (45.5)	51 (69.9)	25 (83.3)	0.056
	wine N (%)	7 (20.6)	17 (21.3)	0.937	20 (20.2)	4 (26.7)	0.516	3 (27.3)	15 (20.5)	6 (20.0)	0.866
	spirits N (%)	27 (79.4)	60 (75)	0.612	74 (74.7)	13 (86.7)	0.312	11 (100)	51 (69.9)	25 (83.3)	0.052
Clinical characteristics											
CP class	A, N (%)	3 (8.8)	11 (13.7)	0.549	10 (10.1)	4 (26.7)	0.088	4 (36.4)	7 (9.6)	3 (10)	0.038[d]
	B, N (%)	17 (50)	30 (37.5)	0.215	39 (39.4)	8 (53.3)	0.307	5 (45.4)	28 (38.4)	14 (46.7)	0.706
	C, N (%)	14 (41.2)	39 (48.8)	0.458	50 (50.5)	3 (20)	0.049[d]	2 (18.2)	38 (52)	13 (43.3)	0.102

The data are expressed as the means ± standard deviations, or medians (25th–75th percentile) unless otherwise noted; active gene refers to the presence of at least one copy of the one gene (*GSTM1* or *GSTT1*);

GSTM1 – glutathione S-transferase M1; GSTT1 – glutathione S-transferase T1; CP – Child–Pugh;

[a]Student's t-test:

[b]Mann–Whitney U test;

[c]Kruskal–Wallis test;

[d]χ^2 test

DOI: https://doi.org/10.2298/SARH251201028N

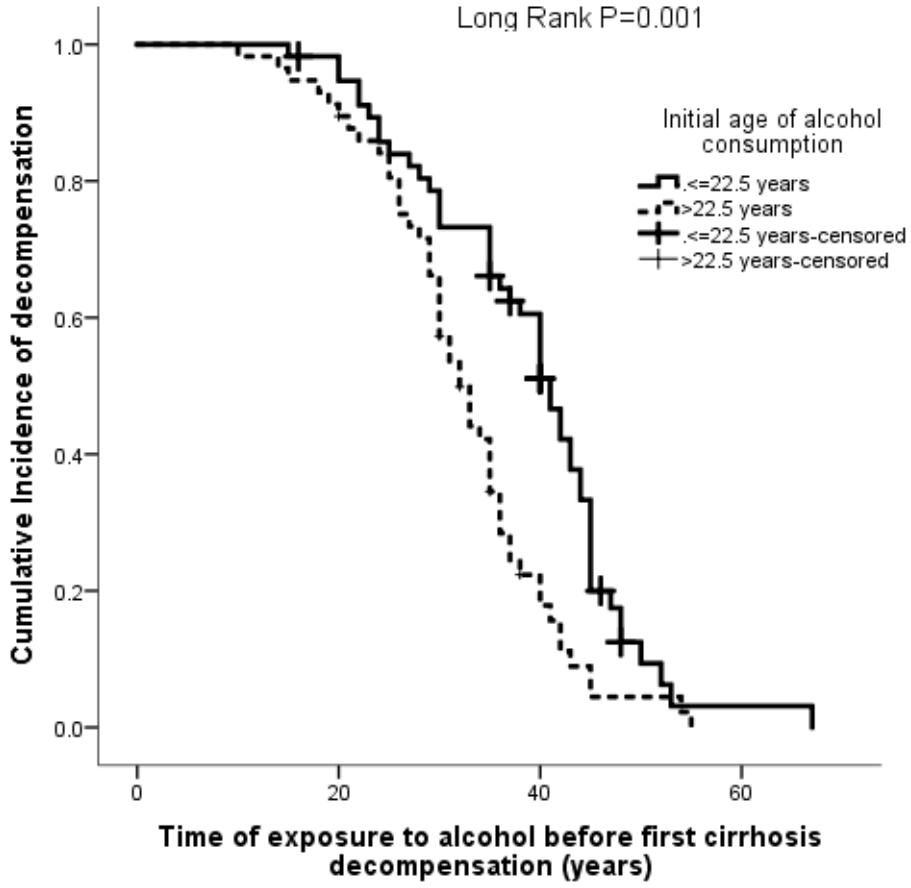

Figure 1. Kaplan–Meier analysis shows that patients who began drinking at ≤ 22.5 years (solid line) developed first cirrhosis decompensation significantly later than those who started after 22.5 years (dashed line) (log-rank p = 0.001); censored cases included nine and five patients, respectively, without decompensation

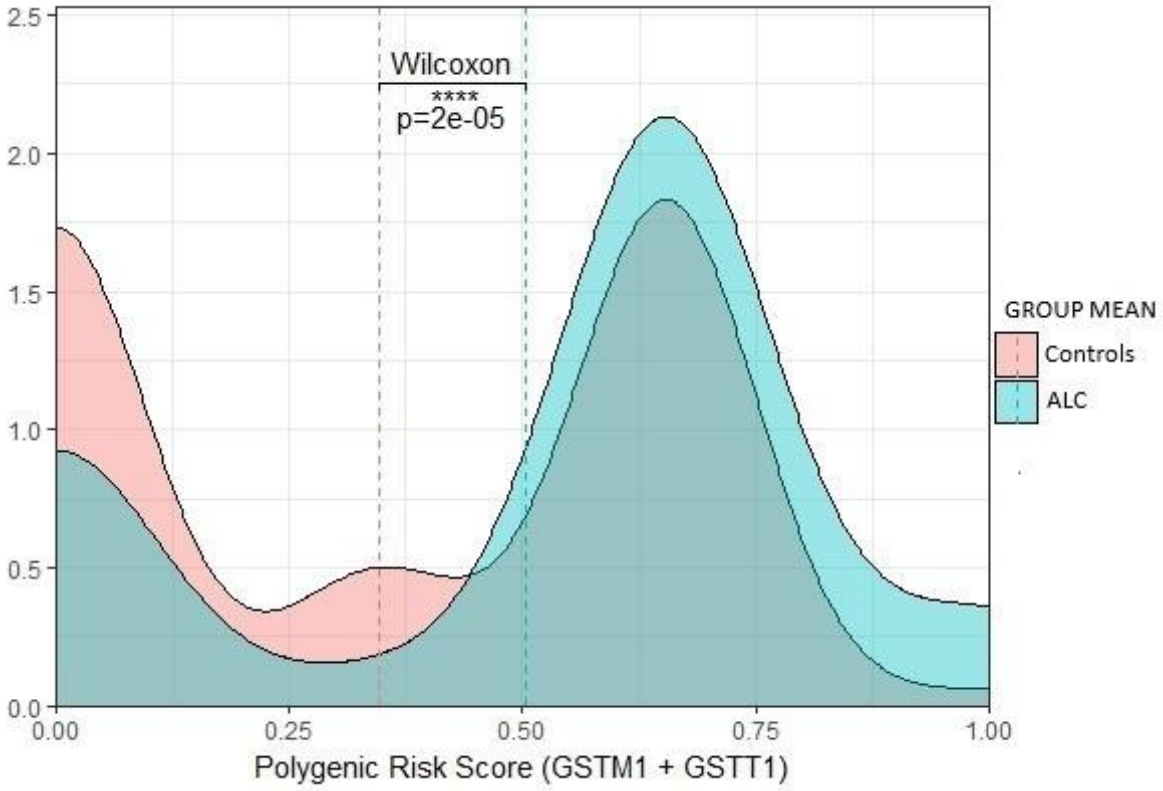

Figure 2. Distribution of *GSTM1* and *GSTT1* deletion variant polygenic risk score (PRS) between controls and alcoholic liver cirrhosis (ALC) patients; ALC patients (blue) had significantly higher PRS values than controls (red) (Wilcoxon test, p = 2e-05); the X-axis shows PRS (0–1), the Y-axis shows density, and dashed lines mark group means

