SOD2 Gene Polymorphism (Ala16Val, rs4880) as a Predictor of Liver Cirrhosis in Chronic Hepatitis C: The Role of Oxidative Stress in Fibrosis Progression

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ABSTRACT

Background and Aim. Oxidative stress is a universal mechanism of hepatocyte damage in chronic hepatitis C (CHC). Manganese superoxide dismutase (SOD2) is the key mitochondrial antioxidant enzyme. The Ala16Val polymorphism (rs4880) affects enzyme import into the mitochondrial matrix. This study aimed to evaluate the association of SOD2 Ala16Val with liver cirrhosis (LC) formation in CHC patients and to assess its relationship with disease severity markers and esophageal varices (EV).

Materials and Methods. 93 CHC patients were enrolled: Group I comprised 48 patients without cirrhosis (F0–F3 by METAVIR) and Group II comprised 45 patients with verified cirrhosis (F4). The control group included 80 healthy individuals matched for age and sex. Genotyping was performed by real-time PCR with TaqMan probes. Esophagogastroduodenoscopy was performed in 67 patients to assess EV grade.

Results. Hardy-Weinberg equilibrium was confirmed in controls (χ^2=0.29; p=0.59). The Val/Val genotype (CC) was found in 42.2% of LC patients vs. 23.8% of controls (OR=2.33; 95% CI 1.07–5.08; χ^2=4.89; p=0.03; RR=1.77; 95% CI 1.10–2.86). Val allele frequency: 64.4% vs. 48.1% (χ^2=6.53; p=0.01). SOD2 demonstrated the highest absolute risk allele gradient (Δ16.3%) among five studied polymorphisms. AUC=0.64 (95% CI 0.54–0.74). Val/Val carriers had significantly higher MELD (17.5±5.2 vs. 14.2±4.1; p<0.05), higher bilirubin, lower platelets, and higher APRI scores. EV grade II–III was found in 73.7% of Val/Val carriers vs. 38.5% of other genotypes (χ^2=5.47; p=0.02; OR=4.44; 95% CI 1.22–16.1).

Conclusion. The SOD2 Ala16Val polymorphism is significantly associated with LC in CHC. The Val/Val genotype predicts more severe cirrhosis and higher frequency of clinically significant EV, confirming oxidative stress as a major driver of fibrosis progression.

Keywords: SOD2, Ala16Val, rs4880, oxidative stress, superoxide dismutase, mitochondria, liver cirrhosis, chronic hepatitis C, fibrogenesis, esophageal varices, portal hypertension

Introduction

Oxidative stress is recognized as one of the universal mechanisms of hepatocyte damage in chronic liver diseases, playing a pivotal role in fibrosis progression from early stages to the formation of cirrhosis [1, 2]. In

chronic hepatitis C, accumulation of reactive oxygen species (ROS) is driven by several synergistic mechanisms: direct effects of viral proteins (Core, NS3, NS5A) on the mitochondrial respiratory chain, activation of NADPH oxidase in Kupffer cells and hepatic stellate cells (HSCs), and induction of cytochrome P450 2E1 (CYP2E1) [3, 4]. The resulting cascade includes lipid peroxidation of membrane phospholipids, damage to mitochondrial and nuclear DNA, oxidative modification of structural proteins, and ultimately, induction of hepatocyte apoptosis through the mitochondrial pathway involving cytochrome c release and caspase activation [5].

Mitochondrial manganese-dependent superoxide dismutase (MnSOD, SOD2) represents the key first-line antioxidant enzyme localized in the mitochondrial matrix. SOD2 catalyzes the dismutation of the superoxide anion radical (O_2^-) to hydrogen peroxide (H_2O_2) and molecular oxygen, preventing accumulation of the most reactive stable ROS [6]. Given that mitochondria are the primary intracellular source of superoxide radicals (accounting for up to 90% of total production), SOD2 efficiency critically determines cellular resistance to oxidative damage.

The Ala16Val polymorphism (rs4880) affects the mitochondrial targeting sequence (MTS) of the SOD2 precursor protein, which governs the efficiency of enzyme import from the cytoplasm into the mitochondrial matrix. The Ala variant (T allele, GCT codon) forms an α-helical conformation of the MTS, ensuring efficient import through the TOM/TIM translocase complex. The Val variant (C allele, GTT codon) disrupts this α-helical structure, reducing transport efficiency and resulting in retention of 30–40% of the precursor protein in the cytoplasm, where it undergoes proteasomal degradation [7, 8]. The functional consequence is a decreased concentration of mature enzyme in the mitochondrial matrix and, accordingly, weakened antioxidant defense.

Degoul et al. (2001) first described the association of this polymorphism with alcoholic liver disease, demonstrating that the Ala/Ala genotype (homozygous for efficient transport) was paradoxically associated with increased cirrhosis risk in alcohol abusers, presumably due to excessive H_2O_2 generation with high SOD2 activity [7]. However, Nahon et al. (2007, 2009) in larger cohort studies established that the Val/Val genotype (reduced activity) was associated with increased risk of cirrhosis and hepatocellular carcinoma in both alcoholic and HCV-related liver disease [9, 10]. These data support a model in which reduced superoxide neutralization capacity leads to chronic oxidative mitochondrial damage and accelerated fibrogenesis.

Data on the association of SOD2 Ala16Val with HCV-associated cirrhosis in Central Asian populations are absent, which defines the relevance of the present study.

The aim of this study was to evaluate the distribution of SOD2 (Ala16Val, rs4880) genotypes in CHC patients with different stages of fibrosis, to determine its association with LC formation, and to investigate the relationship between genotype and clinical markers of disease severity and vascular complications.

Materials and Methods

This study was conducted at the clinic of Andijan State Medical Institute from 2019 to 2024. The protocol was approved by the local ethics committee; all patients provided written informed consent. A total of 93 CHC patients were enrolled: 51 males (54.8%) and 42 females (45.2%), mean age 48.6±11.2 years. The diagnosis was confirmed by detection of anti-HCV antibodies by ELISA and HCV RNA by real-time PCR. Fibrosis stage was verified by transient elastography (FibroScan, Echosens, France).

Group I included 48 (51.6%) patients without cirrhosis (F0–F3 by METAVIR): F0–F1 in 18 (37.5%), F2 in 16 (33.3%), and F3 in 14 (29.2%). Group II comprised 45 (48.4%) patients with verified cirrhosis (F4). Child-Pugh classification in Group II: class A in 21 (46.7%), class B in 17 (37.8%), and class C in 7 (15.5%). The control group consisted of 80 apparently healthy individuals matched for age and sex, without viral hepatitis markers. Exclusion criteria: HBV or HIV co-infection, alcohol abuse (>30 g ethanol/day for males, >20 g for females), autoimmune liver diseases,

hepatocellular carcinoma, and hepatotoxic drug use.

Esophagogastroduodenoscopy (EGD) was performed in 67 patients (35 from Group I and 32 from Group II) to assess esophageal varices (EV) according to the Paquet classification (1983). Patients in Group II were subdivided into EV grade 0–I (n=21) and EV grade II–III (n=24).

Genomic DNA was extracted from venous blood (EDTA) using commercial kits (GeneJET Genomic DNA Purification Kit, Thermo Fisher Scientific). Allelic discrimination of the Ala16Val polymorphism (rs4880) was performed by real-time PCR with allele-specific TaqMan probes on a CFX96 thermal cycler (Bio-Rad). The T allele encodes Ala (efficient mitochondrial transport), while the C allele encodes Val (reduced transport). Each sample was analyzed in duplicate; discordant results triggered a third reaction.

Statistical analysis was performed using SPSS 26.0 and MedCalc 20.0. Pearson's χ^2 test with Yates correction, Fisher's exact test (when expected frequencies <5), odds ratios (OR), relative risks (RR) with 95% confidence intervals (CI) calculated by the Woolf method, and the Cochran-Armitage χ^2 test for linear trend were applied. Hardy-Weinberg equilibrium (HWE) was tested in the control group. ROC analysis with AUC calculation, sensitivity, and specificity was performed. Quantitative variables between subgroups were compared using the Mann-Whitney U test. Differences were considered statistically significant at $p<0.05$.

Results

HWE testing confirmed the expected genotype distribution in the control group ($\chi^2=0.29$; $p=0.59$), indicating sample representativeness and genotyping accuracy. The distribution of genotypes and alleles is presented in Table 1.

Table 1. Distribution of SOD2 (Ala16Val, rs4880) genotypes and alleles

Genotype/ Allele	Controls (n=80)	Group I (n=48)	Group II (n=45)	OR (II vs. Controls)	95% CI	p
TT (Ala/Ala)	22 (27.5%)	12 (25.0%)	6 (13.3%)	0.40	0.15–1.10	0.08
TC (Ala/Val)	39 (48.7%)	23 (47.9%)	20 (44.5%)	0.84	0.41–1.75	0.66
CC (Val/Val)	19 (23.8%)	13 (27.1%)	19 (42.2%)	2.33	1.07–5.08	0.03
T allele (Ala)	51.9%	49.0%	35.6%	0.51	0.30–0.86	0.01
C allele (Val)	48.1%	51.0%	64.4%	1.95	1.16–3.28	0.01

Note: OR and p values are given for comparison of controls vs. Group II.

The frequency of the Val/Val homozygous genotype (CC) was 42.2% in cirrhosis patients, 27.1% in non-cirrhotic patients, and 23.8% in controls. The difference between Group II and controls was statistically significant ($\chi^2=4.89$; $p=0.03$; OR=2.33; 95% CI 1.07–5.08; RR=1.77; 95% CI 1.10–2.86). The Ala/Ala genotype (TT), considered protective, decreased from controls (27.5%) to Group I (25.0%) and further to Group II (13.3%), although the difference with controls reached only a trend level ($p=0.08$). The heterozygous Ala/Val genotype (TC) showed no significant

differences between groups (p=0.66). The genotype distribution is illustrated in Figure 1.

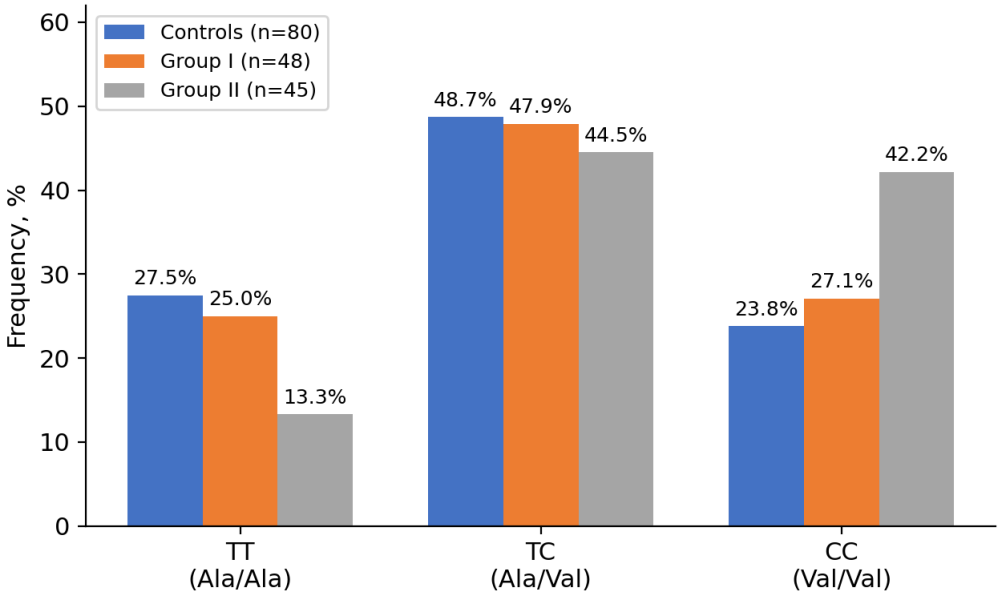

Figure 1. Distribution of SOD2 (Ala16Val) genotypes across study groups

The linear trend of risk allele Val accumulation across the three groups (controls → Group I → Group II: 48.1% → 51.0% → 64.4%) was statistically significant (Cochran-Armitage χ^2 for trend=6.38; p=0.01). The difference between Groups I and II was also significant: Val allele 51.0% vs. 64.4% (χ^2=6.71; p=0.01; OR=1.74; 95% CI 1.08–2.81). The absolute gradient of the Val risk allele (Δ16.3%: from 48.1% in controls to 64.4% in cirrhosis) was the highest among all five studied polymorphisms (TNF-α Δ17.3%, IL28B Δ18.3%, VEGFA Δ16.4%, MMP9 Δ16.3%), indicating a leading role of oxidative stress in CHC progression. Allele frequency dynamics are shown in Figure 2.

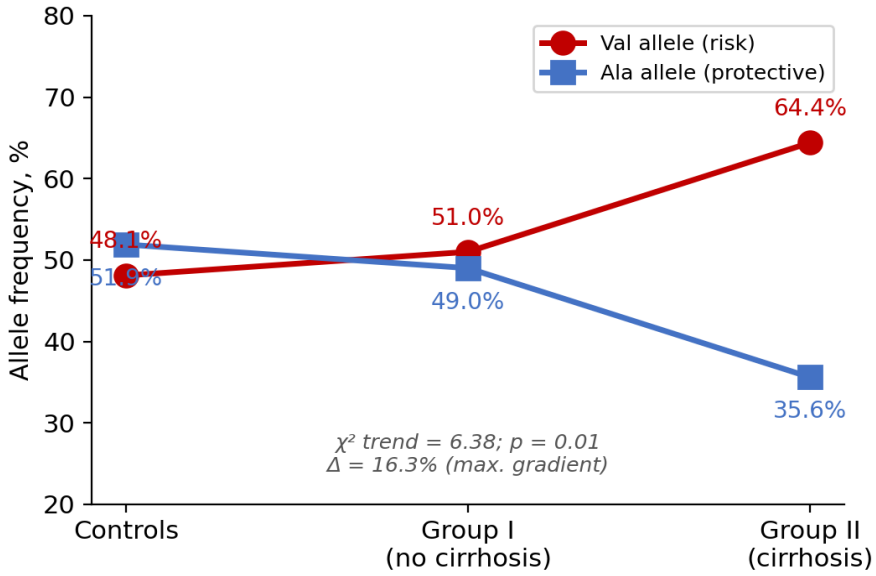

Figure 2. Dynamics of SOD2 (Ala16Val) allele frequencies from controls to cirrhosis (Δ16.3% — maximum gradient)

ROC analysis yielded an AUC of 0.64 (95% CI 0.54–0.74; p=0.02) for SOD2 alone. Sensitivity of the Val/Val genotype for cirrhosis prediction: 42.2%, specificity: 76.2%, PPV: 59.4%, NPV: 57.1%. The moderate AUC is expected for a single polymorphism in a

polygenic disease; however, inclusion of SOD2 in a combined panel of five polymorphisms increases AUC to 0.79 [11].

To evaluate the impact of SOD2 genotype on cirrhosis severity, clinical and laboratory parameters were compared within Group II (Table 2).

Table 2. Clinical and laboratory parameters by SOD2 genotype (Group II, n=45)

Parameter	Ala/Ala + Ala/Val (n=26)	Val/Val (n=19)	p
MELD score	14.2±4.1	17.5±5.2	<0.05
Child-Pugh score	7.1±1.9	8.8±2.7	<0.05
Bilirubin, μmol/L	32.4±14.6	43.1±20.3	<0.05
Albumin, g/L	31.6±5.8	27.2±7.1	<0.05
AST, U/L	54.8±19.4	73.6±28.1	<0.05
Platelets, $\times 10^9$/L	112±38	93±32	<0.05
INR	1.28±0.21	1.47±0.31	<0.05
APRI	1.62±0.74	2.31±1.02	<0.05

Val/Val carriers within Group II demonstrated significantly more severe disease across all major parameters: higher MELD (17.5±5.2 vs. 14.2±4.1; p<0.05), higher Child-Pugh score (8.8±2.7 vs. 7.1±1.9; p<0.05), higher bilirubin (43.1±20.3 vs. 32.4±14.6 μmol/L; p<0.05), higher AST (73.6±28.1 vs. 54.8±19.4 U/L; p<0.05), higher INR (1.47±0.31 vs. 1.28±0.21; p<0.05), and higher APRI (2.31±1.02 vs. 1.62±0.74; p<0.05). Albumin and platelet counts were significantly lower in Val/Val carriers. A graphical comparison is presented in Figure 3.

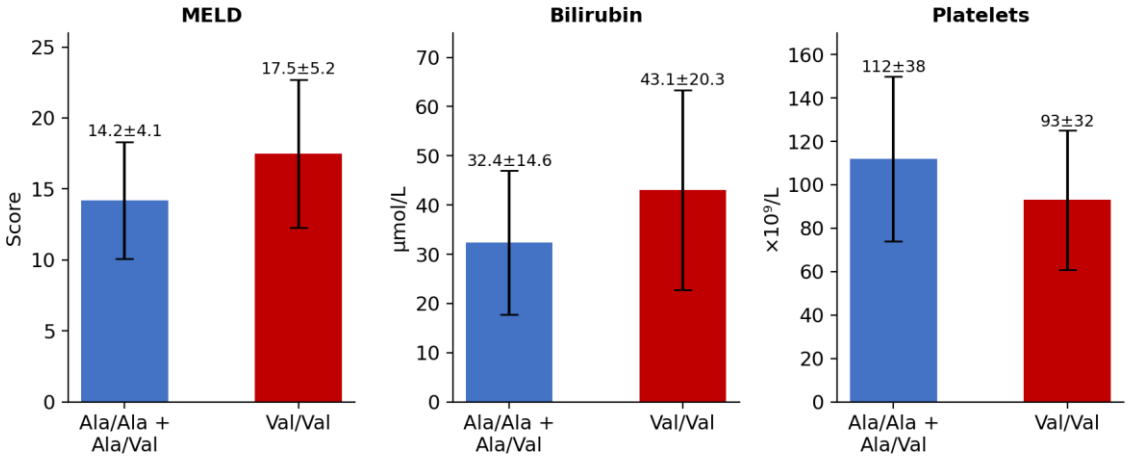

Figure 3. MELD, bilirubin, and platelet counts by SOD2 genotype (Group II)

Association of SOD2 genotype with esophageal varices. To evaluate the association between SOD2 genotype and vascular complications of portal hypertension, the frequency of EV grade II–III within Group II was analyzed by genotype (Table 3).

Table 3. Frequency of esophageal varices grade II–III by SOD2 genotype (Group II, n=45)

Parameter	Ala/Ala + Ala/Val (n=26)	Val/Val (n=19)	OR	95% CI	p
EV grade II–III	10 (38.5%)	14 (73.7%)	4.44	1.22–16.1	0.02
EV grade 0–I	16 (61.5%)	5 (26.3%)	0.23	0.06–0.82	0.02

EV grade II–III was detected in 73.7% of Val/Val carriers compared to only 38.5% of carriers of other genotypes (χ^2=5.47; p=0.02; OR=4.44; 95% CI 1.22–16.1; RR=1.91; 95% CI 1.10–3.33). Thus, the Val/Val genotype is associated not only with cirrhosis formation per se, but also with more severe vascular complications of portal hypertension. This association is illustrated in Figure 4.

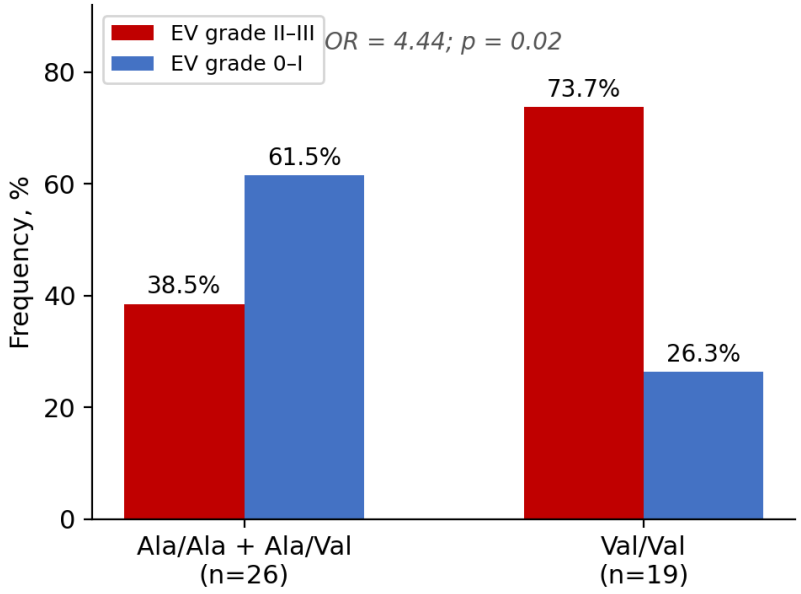

Figure 4. Frequency of esophageal varices grade II–III by SOD2 genotype (Group II)

Discussion

The present findings confirm the central role of oxidative stress in HCV-associated fibrosis progression and, for the first time, demonstrate the association of SOD2 Ala16Val polymorphism with liver cirrhosis in a Central Asian population. Our results are consistent with those of Nahon et al. (2009), who reported predominance of the Val/Val genotype among patients with alcoholic cirrhosis [10], and extend these observations to HCV-induced liver damage.

The pathogenetic mechanism underlying the Val/Val–cirrhosis association is well characterized. The Val/Val genotype reduces SOD2 import efficiency into the mitochondrial matrix by 30–40%, leading to superoxide radical accumulation and its conversion to more aggressive ROS, including peroxynitrite ($ONOO^-$) and hydroxyl radical (•OH) [7, 8]. Chronic ROS accumulation in hepatocyte mitochondria triggers a vicious cycle: oxidative damage to mitochondrial DNA disrupts the respiratory chain, further increasing superoxide production. The result is massive hepatocyte apoptosis, release of danger-associated molecular patterns (DAMPs), and activation of HSCs — the principal collagen-producing cells in the fibrotic liver [3, 5].

A critically important finding is the maximum absolute gradient of the Val risk allele (Δ16.3%) among all five studied polymorphisms. This observation suggests that

among the multiple pathogenetic pathways of fibrogenesis (inflammation, impaired antiviral immunity, pathological angiogenesis, extracellular matrix remodeling), oxidative stress may contribute most substantially to CHC progression toward cirrhosis. This finding has direct therapeutic implications: Val/Val carriers may benefit from supplemental antioxidant therapy (N-acetylcysteine, S-adenosylmethionine, vitamin E) in addition to antiviral treatment.

The correlation between Val/Val genotype and EV severity (OR=4.44) represents a novel and clinically significant result. This can be explained by the fact that oxidative stress damages not only hepatocytes but also sinusoidal endothelial cells. Oxidative modification of sinusoidal endothelial cells leads to loss of fenestrations, formation of a continuous basement membrane (sinusoidal capillarization), and increased intrahepatic vascular resistance — the central mechanism of portal hypertension [12]. Furthermore, decreased nitric oxide (NO) bioavailability due to its inactivation by superoxide impairs the vasodilatory function of the endothelium, exacerbating portal hypertension.

Comparison of our results with those of Degoul et al. (2001), who described an association of the Ala/Ala genotype with alcoholic cirrhosis, requires comment [7]. These authors hypothesized that excessive H_2O_2 production with high SOD2 activity might amplify oxidative damage. However, subsequent studies by Nahon et al. (2007, 2009) did not confirm this hypothesis and showed the opposite relationship, consistent with our data [9, 10]. The discrepancy may reflect differences in etiology (alcohol vs. HCV), sample sizes, and stratification methods. In the context of HCV infection, where viral proteins directly damage the mitochondrial respiratory chain, reduced SOD2 activity (Val/Val genotype) represents a more significant risk factor.

Study limitations include the single-center design, relatively small sample size, and lack of direct SOD2 activity measurement or ROS levels in serum or liver tissue. Functional confirmation of the genetic association by measuring oxidative stress markers (malondialdehyde, 8-hydroxy-2-deoxyguanosine, total antioxidant capacity) is a promising direction for future research. Additionally, multicenter validation with inclusion of diverse Central Asian ethnic groups is warranted.

Conclusions

1. The SOD2 Ala16Val (rs4880) polymorphism is significantly associated with liver cirrhosis in CHC: the Val/Val genotype predominates in cirrhosis (42.2% vs. 23.8% in controls; OR=2.33; 95% CI 1.07–5.08; p=0.03).
2. The Val risk allele frequency demonstrates a significant linear trend from controls to cirrhosis (χ^2 for trend=6.38; p=0.01) with the maximum absolute gradient (Δ16.3%) among five studied polymorphisms, indicating a leading role of oxidative stress in fibrosis progression.
3. Val/Val carriers among cirrhosis patients have significantly more severe disease as assessed by MELD, Child-Pugh, and laboratory markers of hepatic insufficiency (bilirubin, albumin, platelets, INR, APRI).
4. The Val/Val genotype is associated with a significantly higher frequency of EV grade II–III (73.7% vs. 38.5%; OR=4.44; p=0.02), confirming the role of oxidative endothelial damage in portal hypertension progression.
5. SOD2 Ala16Val genotyping is recommended for inclusion in combined genetic panels for LC prediction in CHC. Val/Val carriers may benefit from supplemental antioxidant therapy.

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