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EDITORIAL

Liver enzymes, metabolomics and genome-wide association studies: From systems biology to the personalized medicine

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Abstract

For several decades, serum levels of alanine (ALT) and aspartate (AST) aminotransferases have been regarded as markers of liver injury, including a wide range of etiologies from viral hepatitis to fatty liver. The increasing worldwide prevalence of metabolic syndrome and cardiovascular disease revealed that transaminases are strong predictors of type 2 diabetes, coronary heart disease, atherothrombotic risk profile, and overall risk of metabolic disease. Therefore, it is plausible to suggest that aminotransferases are surrogate biomarkers of "liver metabolic functioning" beyond the classical concept of liver cellular damage, as their enzymatic activity might actually reflect key aspects of the physiology and pathophysiology of the liver function. In this study, we summarize the background information and recent findings on the biological role of ALT and AST, and review the knowledge gained from the application of genome-wide approaches and "omics" technologies that uncovered new concepts on the role of aminotransferases in human diseases and systemic regulation of metabolic functions. Prediction of biomolecular interactions between the candidate genes recently discovered to be associated with plasma concentrations of liver enzymes showed interesting interconnectivity nodes, which suggest that regulation of aminotransferase activity is a complex and highly regulated trait. Finally, links between aminotransferase genes and metabolites are explored to understand the genetic contributions to the metabolic diversity.

Key words: Transaminases; Aminotransferases; Alanine-aminotransferase; Aspartate-aminotransferase; Glutamate-oxalacetate transaminase; Glutamate-pyruvate transaminase; Glutamic acid; Metabolism; Nonalcoholic fatty liver; Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Gene variants; Single nucleotide polymorphisms; *PNPLA3*; Genetics; Metabolomics; Metabolic syndrome; Systems biology

Core tip: Genomic, transcriptomic, proteomic, and metabolomic information has changed the classical conception of the meaning that serum concentrations of alanine- (ALT) and aspartate (AST) aminotransferase are merely indicators of hepatocyte membrane disruption. It has given way to a more complex and interconnected view of the importance of liver transaminases in the regulation of systemic metabolic function.

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INTRODUCTION

For several decades, serum levels of alanine (ALT) and aspartate (AST) aminotransferases have been regarded as markers of liver injury, including a wide range of etiologies from viral hepatitis to fatty liver[1]. The first report of the role of liver transaminases in the prediction of liver cellular damage was published in 1955 by Molander and colleagues, after noticing that the levels of glutamic oxalacetic transaminase (GOT or AST) were elevated after acute myocardial infarction[2]. It is noteworthy to mention that high serum GOT activity observed in patients with myocardial infarction and acute heart failure is mostly attributed to the accompanying acute central necrosis of the liver associated with circulatory changes, as elegantly demonstrated by Killip *et al*[3].

Although the chemical reaction mediated by a transaminase was initially described in 1950[4], measurement of ALT and AST enzymatic activity in circulation is still the most commonly used biochemistry test in clinical practice, when the aim is to evaluate putative liver injury[5]. Notably, while the correlation between the degree of hepatocyte injury and amininotransferase levels is poor[1], it is accepted that blood levels of ALT and AST are a consequence of the liver cell membrane damage, with the subsequent leakage of intracellular enzymes into the circulation, especially the cytosolic ones[6,7].

The increasing worldwide prevalence of metabolic syndrome (Met Synd) and cardiovascular disease (CVD) has revealed that transaminases are relaible predictors of the individual components of this very complex trait, including type 2 diabetes[8] and decreased hepatic insulin sensitivity[9], coronary heart disease[10], atherothrombotic risk profile[11], and overall risk of cardiovascular[12] and metabolic disease[13].

Therefore, routine testing of aminotransferases ALT and AST, initially regarded as markers of liver injury, is increasingly being considered as an indicator of the "liver metabolic function"[14]. Based upon this evidence, it is reasonable to speculate that the ALT and AST enzymatic activity measured in circulation actually reflects relevant aspects of the physiology and pathophysiology of the liver function beyond hepatocyte membrane disruption.

In this study, we summarize the background information and recent findings on the biological function of ALT and AST, and review the knowledge gained from the application of genome-wide approaches and "omics" technologies that uncovered new concepts of the role of aminotransferases in human diseases and systemic regulation of metabolic functions.

BRIEF OVERVIEW OF ALT AND AST GENE AND PROTEIN FUNCTION: A PIVOTAL ROLE IN GLUCOSE METABOLISM

Aminotransferases are enzymes that catalyze the transfer of an alpha-amino group from an amino acid to an alpha-keto acid. They share certain mechanistic features with other pyridoxal-phosphate-dependent enzymes. With respect to the domain features, aminotransferases are grouped into different classes, including class Ⅰ, Ⅱ, Ⅲ, Ⅳ and Ⅴ. ALT and AST belong to the class- I pyridoxal-phosphate-dependent aminotransferase, which comprises 11 proteins in the human proteome, as shown in Table 1. In this review, we will refer to aminotransferase genes as to *GPT* and *GOT,* including their related isoforms.

While there are two isoforms of human ALT, namely ALT1 and ALT2, when referring to the protein, we will use the ALT name. The gene that encodes for the cytosolic alanine aminotransaminase 1 protein (ALT1), also known as glutamate-pyruvate transaminase 1 (*GPT1* or formaly *GPT*), is located in chromosome 8 (8q24.3) and has 11 exons.

This enzyme catalyzes the reversible transamination between alanine and 2-oxoglutarate to generate pyruvate and glutamate, playing a key role in the intermediary metabolism of glucose and amino acids. *ALT1* is expressed in liver, kidney, heart, and skeletal muscle, and at moderate levels in the adipose tissue[15].

ALT2 is encoded by a different gene (GPT2), located in chromosome 16 (16q12.1). The *GPT2* mRNA is expressed at high levels in muscle, fat, kidney, and brain, and at lower levels in liver and breast[16]. As, in some studies, neither liver nor kidney showed ALT2 expression[17], this issue clearly requires further investigation.

According to available evidence, ALT1 and ALT2 seem to have not only different tissue source, but cellular localization as well, suggesting a dissimilar biological meaning in the context of acute or chronic liver disease pathogenesis. Figure 1 depicts a schematic representation of ALT1 and ALT2 protein localization at the cellular level. For example, in the liver, ALT1 localizes only in the cytosol and endoplasmic reticulum, with no presence in mitochondria[18]. Conversely, ALT2 is preferably localized in the mitochondrial matrix (Figure 1). Thus, while current

Table 1 List of enzymes of the Class- I pyridoxal-phosphate-dependent aminotransferase family: Evidence from the human proteome

Protein name	Gene name	Chromosome	Number of Isoforms
1-aminocyclopropane-1-carboxylate synthase-like protein 1	*ACCS*	11p11.2	1
Alanine aminotransferase 1	*GPT1*	8q24.3	1
Alanine aminotransferase 2	*GPT2*	16q11.2	2
Aspartate aminotransferase, cytoplasmic	*GOT1*	10q24.2	1
Aspartate aminotransferase, mitochondrial	*GOT2*	16q21	2
Kynurenine/alpha-aminoadipate aminotransferase, mitochondrial	*AADAT*	4q33	2
Kynurenine--oxoglutarate transaminase 1	*CCBL1*	9q34.11	3
Kynurenine--oxoglutarate transaminase 3	*CCBL2*	1p22.2	3
Probable inactive 1-aminocyclopropane-1-carboxylate synthase-like protein 2	*ACCSL*	11p11.2	1
Putative aspartate aminotransferase, cytoplasmic 2	*GOT1L1*	8p11.23	1
Tyrosine aminotransferase	*TAT*	16q22.2	1

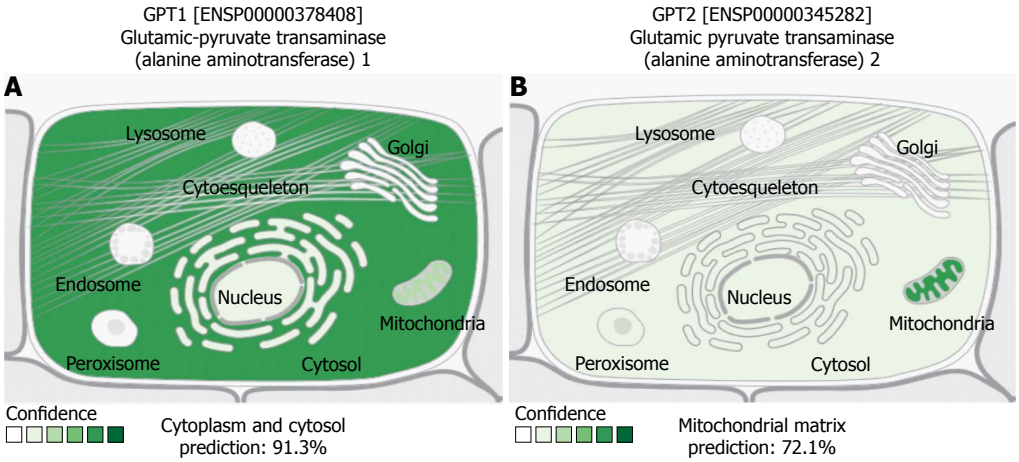

Figure 1 Schematic representation of different localizations of ALT1 (GPT1) and ALT2 (GPT2) proteins at the cellular level. Prediction was performed by the open access web resource "COMPARTMENTS" available at http://compartments.jensenlab.org, which predicts protein localization according to information extracted from different databases, including UniProtKB, as well as cellular component ontologies visualized by the Gene Ontology Consortium. The program generates unified confidence scores of the localization evidence; confidence scale is color coded, ranging from light green (1) indicating low confidence, to dark green (5), corresponding to high confidence, with absence of localization evidence depicted in white (0). The evidence score is expressed in %.

evidence from human studies is scarce, it is plausible to suggest that ALT1 and ALT2 might reflect hepatocyte membrane disruption and mitochondrial dysfunction, respectively. Unfortunately, current biochemical tests aimed at measuring ALT in circulation are incapable of identifying the cellular source. Nevertheless, while routine assessment of ALT activity does not discriminate between ALT isoforms, a recent study demonstrated that most of the activity in circulation is given by ALT1[19].

In addition, supporting the notion that elevation of ALT levels does not necessarily denote hepatocellular damage, Kechagias *et al*[20] showed that fast-food-based hyper-alimentation in combination with a sedentary lifestyle, when followed for four weeks, was associated with pathological serum ALT levels. Notably, the authors showed that the significant elevation of aminotransferases (up to 447 U/L) associated with the hyper-alimentation regimen were not related to the development of liver steatosis[20]. This clinical finding reinforces the hypothesis that an increase in the ALT enzymatic activity is an adaptive response to the liver metabolic demands[14]. Table 2 summarizes the main features of ALT1 and ALT2, including novel aspects on their biological function and gene regulation, such as modulation of *GPT1* by miR-122 to enhance ALT enzymatic activity, as recently reported by our group[21].

Glutamate-oxalacetate transaminase (GOT) is a pyridoxal phosphate-dependent enzyme that exists in cytoplasmic and mitochondrial forms, GOT1 and GOT2, respectively. As previously noted, the two enzymes belong to the class- I pyridoxal-phosphate-dependent aminotransferase family, and are homodimeric, showing close homology.

The gene that encodes for the soluble GOT1 (*GOT1*) is located in chromosome 10 (10q24.2), while the one that encodes for the mitochondrial GOT2 (*GOT2*) is located in chromosome 16 (16q21). GOT1 is an important regulator of glutamate levels, as it is involved in the biosynthesis of L-glutamate from L-aspartate or L-cysteine. The catalytic unit of GOT1 is responsible for the following reactions: L-aspartate + 2-oxoglutarate = oxaloacetate + L-glutamate and L-cysteine + 2-oxoglutarate = mercaptopyruvate + L-glutamate. GOT1 aliases are cysteine aminotransferase and transaminase A. In addition, the aspartate aminotransferase activity is involved in hepatic glucose synthesis during development and in adipocyte

Table 2 Comparison of biological and protein function of ALT1 and ALT2: background information and recent findings

Features and function	Glutamic-Pyruvate Transaminase 1 (Alanine Aminotransferase 1) GPT1	Glutamic pyruvate transaminase 2 (alanine aminotransferase) 2 GPT2
Gene and protein Id in data-bases	Entrez Gene: 2875 Ensembl gene: ENSG00000167701 UniProtKB: P24298	Entrez gene: 84706 Ensemble: ENSG00000166123 UniProtKB: Q8TD30
Genomic location	Entrez Gene cytogenetic band: 8q24.3	Entrez gene cytogenetic band: 16q12.1
Number of gene transcripts	7 transcripts (splice variants), 28 exons on the forward strand	5 transcripts (splice variants), 26 exons on the forward strand
Variation	*GPT1* has 210 SNPs	*GPT2* has 819 SNPs
Orthologues	*GPT1* has 69 orthologues in Ensembl	*GPT2* has 63 orthologues in Ensembl
Regulation	There are 2 regulatory elements located in the region of *GPT1* gene miR-122 may interact with *GPT1* at multiple sites of the coding region to enhance translation[21] Microsomal triglyceride transfer protein inhibition augments plasma ALT/AST levels in response to endoplasmic reticulum stress[66] *GPT1*, but not *GPT2* promoter is induced by PPAR agonists[67] ALT1 catalytic activity is inhibited by the effect of glycation[68]	There are 13 regulatory elements located in the region of *GPT2* *GPT2* promoter has a putative ATF4 (Activating transcription factor 4 binding site[69] *GPT2* is regulated by androgens in non-hepatic tissues[70]
Protein features	Size: 496 amino acids; 54637 Da Cofactor: Pyridoxal phosphate Subunit: Homodimer	Size: 523 amino acids; 57904 Da Cofactor: Pyridoxal phosphate Subunit: Homodimer (By similarity)
Cellular localization in human cells[1]	Cytosol of hepatocytes[18]	ER and mitochondrial fraction[18]
Measurement in plasma (catalytic activity)	Represents 90% of total ALT in circulation[17,18]	Represents 10% of total ALT in circulation[17,18]
Tissue expression in humans	Evidence: WB: Liver and kidney[18] Evidence: NB: GPT mRNA is moderately expressed in kidney, liver, heart, and fat[15]	Evidence: WB and IHQ (protein): Pancreas (islets of Langerhans), brain, adrenal gland, skeletal muscle, heart (cardiomyocytes)[18] Evidence: NB: mRNA is expressed at high levels in muscle, fat, kidney, and brain, and at lower levels in liver and breast[15]
Tissue expression in rodents	Evidence: NB (mRNA): Highly expressed in liver and moderately expressed in white adipose tissue (WAT), intestine, and colon[71]	Evidence: NB (mRNA): muscle, liver, and white adipose tissue (WAT), at moderate levels in brain and kidney, and at a low level in heart[71] Gene expression analysis suggests a sex-dependent difference in *GPT2*-mRNA in the liver and muscle[15] Hepatic and muscle ALT2 protein activity was higher in males than in females; while no sex-dependent difference was noted in the liver for ALT1, it appears 20% higher in muscle in females[15]
Biological meaning and metabolic function	ALT1 contributes to "basal" serum ALT activity, most likely associated with normal cell turnover in liver and other tissues that would release ALT1 into the circulation[15,17-19]	Generation of pyruvate for gluconeogenesis under stressful living conditions, such as starvation[18] ALT2 is involved in the metabolic adaptation of the cell to stress[69] ALT2 is associated with a liver progluconeogenic metabolic adaptive response without hepatocellular necrosis after exposure to dexamethasone[72] ALT2 may participate in the generation of pyruvate and glyceroneogenesis, contributing to the homeostasis of fatty acid metabolism and storage[16]
Biological meaning in human disease	NAFLD: ALT1 represented 94% of total ALT levels in circulation[19] HCV: High levels in circulation of ALT1 (about 5-fold increase as compared to the controls)[19] Ultra-endurance exercise: no significant changes after exercise[19]	NAFLD: ALT2 represented 6% of total ALT levels in circulation[19] HCV: Moderate levels in circulation of ALT1 (about 2.5 fold increase as compared to the controls)[19] Ultra-endurance exercise: High levels in circulation of ALT2 (about 2-fold increase as compared to the baseline conditions)[19]
Biological meaning in experimental models of disease	NAFLD (*ob/ob*): Compared to the normal liver of lean mice, the expression of *GPT1* mRNA remained unchanged[71] Both ALT1 and ALT2 increased in the liver of mice induced liver steatosis by a deficient methionine-choline diet[73]	NAFLD (*ob/ob*): Compared to the normal liver of lean mice, the expression of *GPT2* mRNA was elevated by about 2-fold, suggesting ALT2 induction during fatty liver[71]

[1]Cellular localization differs among species. ER: Endoplasmic reticulum; WB: Western blotting; IHQ: Immunohistochemistry; NB: Northern blot analysis; HCV: Hepatitis C virus; NAFLD: Non-alcoholic fatty liver disease.

Table 3 Evidence from genome-wide association studies on the heritability of circulating levels of alanine-aminotransferase and aspartate-aminotransferase

Ref.	Number of participants/ study design	GWAS strategy (genotyping)	Number of variants	Phenotype	Identified locus
Chambers *et al*[36]	*n* = 61089 Population-based Adults	Affymetrix, Illumina and perlegen sciences arrays	About 2.6 million directly genotyped or imputed autosomal SNPs	Plasma levels of ALT	*HSD17B13, MAPK10, TRIB1, CPN1, PNPLA3, SAMM50*
Yuan *et al*[74]	Initial study *n* = 7715 Replication *n* = 704 Population-based Adults	Affymetrix	-	Plasma levels of ALT	*CHUK, PNPLA3, SAMM50, CPN1*
Park *et al*[75]	*n* = 532	Illumina HumanOmni1-Quad BeadChip	747076 SNPs	Plasma levels of ALT	*ST6GALNAC3, MMADHC, CCDC102B, RGS5, BRD7, GALNT13, SIRPA, CD93, SLC39A11, ADAMTS9, CELF2*
	Population-based Children			Plasma levels of AST	*CYB5APS, CELF2, GOT1, ST6GALNAC3, ADAMTS9, THSD7B, EIF4A1P1, ROBO1, THSD7B*
Shen *et al*[76]	*n* = 866	Affymetrix GeneChip Human Mapping 500 K Array set	500568 SNPs	Plasma levels of AST	*GOT1*
	Population-based adults				

GWAS: Genome-wide association studies; SNPs: Single nucleotide polymorphisms; ALT: Alanine-aminotransferase; AST: Aspartate-aminotransferase.

glyceroneogenesis.

GOT2 catalyzes the irreversible transamination of the L-tryptophan metabolite L-kynurenine to form kynurenic acid (L-kynurenine + 2-oxoglutarate = 4-(2-aminophenyl)-2,4-dioxobutanoate + L-glutamate) and the reversible transamination of L-aspartate + 2-oxoglutarate = oxaloacetate + L-glutamate. GOT2 plays a key role in amino acid metabolism and the metabolite exchange between mitochondria and cytosol. It also facilitates cellular uptake of long-chain free fatty acids. Of note, GOT2 is also known by the following aliases: fatty acid-binding protein, kynurenine aminotransferase 4, plasma membrane-associated fatty acid-binding protein and kynurenine-oxoglutarate transaminase Ⅳ.

GENETIC HERITABILITY ON THE SERUM LEVELS OF LIVER AMINOTRANSFERASES: EVIDENCE FROM GENOME-WIDE ASSOCIATION STUDIES

The serum level of transaminases is highly variable and is affected by a myriad of factors, including demographic ones, such as sex, age and ethnicity; anthropometric features, such as waist circumference and body mass index; and environmental factors, such as alcohol consumption[22]. Serum level of transaminases is also subject to diurnal variation[23,24].

In addition, it is known that ALT and AST concentrations in circulation are heritable[25]. In fact, studies have shown that ALT and AST levels are highly heritable, with additive genetic effects accounting for 48% and 32% of the variation, respectively[26]. Furthermore, results from a population-based study in twins showed that the heritability for ALT and AST is not gender specific[27].

To examine the genetic influence on plasma/serum levels of aminotransferases, four genome-wide association studies (GWAS) exploring a large number of SNPs were conducted in different populations around the world, as summarized in Table 3. Findings of these studies have shed light on new interesting candidate genes associated with liver enzymes, including the largely replicated *PNPLA3* (patatin-like phospholipase domain containing 3) gene that is not only associated with nonalcoholic fatty liver disease (NAFLD)[28] and nonalcoholic steatohepatitis (NASH)[29,30], but also a wide spectrum of chronic liver diseases, as recently highlighted[31], including alcoholic liver disease[32], viral hepatitis C[33] and B[34] and hepatocellular carcinoma[35]. Interestingly, the rs738409 located in *PNPLA3* reached the most significant p value for association with liver enzyme levels (1.2×10^{-45}) in the larger GWAS performed in Caucasians[36]. As expected, most of the associated variants with liver enzyme levels are either intergenic or intronic single nucleotide polymorphisms (SNPs), and the loci or nearest gene in which they are located has either an unknown function or a biological role not known to be associated with liver enzymes. Moreover, with the exception of the rs738409[37-39], there is presently no evidence supporting a putative pathogenic, damaging or deleterious effect of the discovered variants, either on the protein function or in the regulation of the related gene.

A detailed overview of the associated variants with aminotransaminase levels, their main features, and the biological role of the gene where are they located is

Table 4 Summary of the variants associated with alanine-aminotransferase and aspartate-aminotransferase levels in population-based genome-wide association studies: Biological function and variants characteristics

Variant ID	Variant features	Significant *P* value for GWAS association	Gene or nearest gene	Reported biological function of the associated locus
ALT				
rs6834314	Intergenic	3.1×10^{-9}	*HSD17B13/ MAPK10*	Oxidoreductase involved in the metabolism of steroid hormones, prostaglandins, retinoids, lipids and xenobiotics A member of the MAP kinase family
rs2954021	Intron variant	5.3×10^{-9}	*TRIB1*	Involved in protein amino acid phosphorylation and controlling mitogen-activated protein kinase cascades. Potent negative regulator of MAPK pathways influencing apoptosis. Regulates hepatic lipogenesis and very low density lipoprotein production
rs10883437	Intergenic	4.0×10^{-9}	*CPN1*	A plasma metallo-protease that cleaves basic amino acids from the C terminal of peptides and proteins
rs11597390	intergenic	2.9×10^{-8}		
rs738409	Missense p.Ile148Met	1.2×10^{-45}	*PNPLA3*	Acylglycerol O-acyltransferase and triacylglycerol lipase that mediates triacylglycerol hydrolysis
rs2281135	Intron variant	8.2×10^{-12}		
rs3761472	Missense p.Asp110Gly	3.7×10^{-29}	*SAMM50*	Component of the sorting and assembly machinery (SAM) of the mitochondrial outer membrane
rs2143571	Intron variant	9.4×10^{-7}		
rs11597086	Non coding exon variant	3.6×10^{-7}	*CHUK*	Member of the serine/threonine protein kinase family; a component of a cytokine-activated protein complex that is an inhibitor of the essential transcription factor NF-kappa-B complex
rs11591741	Intron variant	4.5×10^{-7}		
rs4949718	Intron variant	1.87×10^{-7}	*ST6GALNAC3*	Transfer sialic acids from CMP-sialic acid to terminal positions of carbohydrate groups in glycoproteins and glycolipids
rs17801127	Intergenic	2.37×10^{-7}	*MMADHC*	Mitochondrial protein that is involved in an early step of vitamin B12 metabolism
rs1539893	Intron variant	3.40×10^{-6}	*CCDC102B*	Unknown
rs12035879	Intron variant	3.97×10^{-6}	*RGS5*	Member of the regulators of G protein signaling (RGS) family
rs9941219	Intergenic	4.06×10^{-6}	*BRD7*	Member of the bromodomain-containing protein family
rs731660	Intergenic			
rs12621256	Intron variant	4.36×10^{-6}	*GALNT13*	Member of the glycosyltransferase 2 family; catalyzes the initial reaction in oligosaccharide biosynthesis; neurons cell biogenesis
rs6035126	Intergenic	4.94×10^{-6}	*SIRPA*	Receptor-type transmembrane glycoproteins involved in the negative regulation of receptor tyrosine kinase-coupled signaling processes
rs13433286	Intergenic			
rs844917	Intergenic	5.64×10^{-6}	*CD93*	Cell-surface glycoprotein and type I membrane protein
rs844914	Intergenic	5.98×10^{-6}		
rs903107	Intron variant	6.11×10^{-6}	*SLC39A11*	Mediates zinc uptake
rs80311637	Missense p.Val653Met	7.18×10^{-6}	*ADAMTS9*	Disintegrin and metalloproteinase with thrombospondin motifs
rs596406	Intron variant	9.18×10^{-6}	*CELF2*	RNA-binding protein implicated in the regulation of several post-transcriptional events
AST				
rs11597390	Intergenic	0.0009	*CHUK*	Explained previously
rs2281135	Intron variant	5.7×10^{-6}	*PNPLA3*	Explained previously
rs862946	Intergenic	2.41×10^{-7}	*CYB5AP5*	Pseudogene
rs596406	Intron variant	3.69×10^{-7}	*CELF2*	Explained previously
rs76850691	Missense p.Gln349Glu	8.55×10^{-7}	*GOT1*	Biosynthesis of L-glutamate from L-aspartate or L-cysteine
rs17109512	Intergenic	2.80×10^{-14}		
rs4949718	Intron variant	1.49×10^{-6}	*ST6GALNAC3*	Explained previously
rs80311637	Missense p.Val1597Met	1.85×10^{-6}	*ADAMTS9*	Explained previously
rs892877	Intron variant	3.75×10^{-6}	*THSD7B*	Unknown
rs984295	Intron variant	5.86×10^{-6}		
rs457603	Intergenic	4.57×10^{-6}	*EIF4A1P1*	Pseudogene
rs452621	Intergenic			
rs7617400	Intron variant	6.16×10^{-6}	*ROBO1*	Neuronal development
rs11924965	Intron variant			
rs7644918	Intron variant			

HSD17B13: Hydroxysteroid (17-beta) dehydrogenase 13; *MAPK10*: Mitogen-activated protein kinase 10; *TRIB1*: Tribbles pseudokinase 1; *CPN1*: Carboxypeptidase N, polypeptide 1; PNPLA3: Patatin-like phospholipase domain containing 3; *SAMM50*: Sorting and assembly machinery component; CHUK: Conserved helix-loop-helix ubiquitous kinase; ST6GALNAC3: ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 3; MMADHC: Methylmalonic aciduria (cobalamin deficiency) cblD type, with homocystinuria; CCDC102B: Coiled-Coil Domain Containing 102B; RGS5: Regulator of G-protein signaling 5; *BRD7*: Bromodomain containing 7; *GALNT13*: Polypeptide N-acetylgalactosaminyltransferase 13; *SIRPA*: Signal-regulatory protein alpha; *CD93*: *CD93* molecule; *SLC39A11*: Solute carrier family 39, member 11; *ADAMTS9*: ADAM metallopeptidase with thrombospondin type 1 motif, 9; *CYB5AP5*: Cytochrome b5 type A (microsomal) pseudogene 5; *CELF2*: CUGBP, Elav-like family member 2; *GOT1*: Glutamic-oxaloacetic transaminase 1, soluble; *THSD7B*: thrombospondin, type I, domain containing 7B; *EIF4A1P1*: Eukaryotic translation initiation factor 4A1 pseudogene 1; *ROBO1*: Roundabout, axon guidance receptor, homolog 1. Biological function was extracted from Gene Atlas (http://genatlas.medecine.univ-paris5.fr).

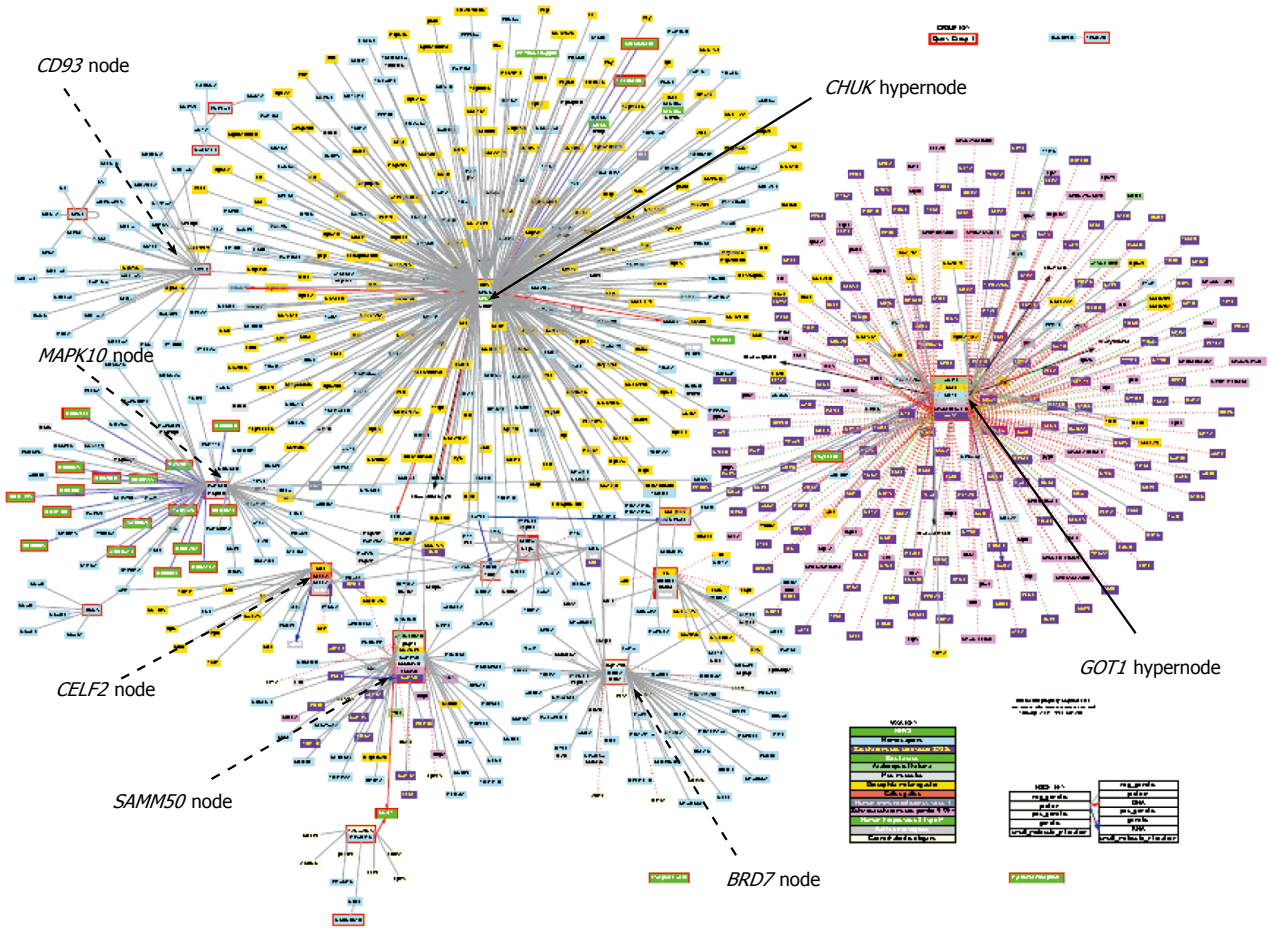

Figure 2 Visualization of biomolecular interactions among associated loci with serum levels of alanine-aminotransferase and aspartate-aminotransferase in published genome-wide association studies. Prediction was based on the Cognoscente program, freely available at the web-based submission portal: http://vanburenlab.tamhsc.edu/cognoscente.html. The interaction network image shows 828 nodes with different levels of complexity; black arrows indicate the major nodes. Additional interconnectivity nodes of importance (highlighted in black dashed arrows) are *SAMM50*, *CD93* (highly connected with *CHUK*), *MAPK10*, *CELF2* and *BRD7*. Prediction by Cognoscente supports multiple organisms in the same query, as well as gene-gene, gene-protein, protein-RNA and protein-DNA interactions, and multi-molecule queries[77]. The input list was based on the gene list presented in Table 4, while the graph depicts known interactions the query list members.

shown in Table 4.

SYSTEMS BIOLOGY APPROACHES TO EXPLORING A PUTATIVE CONNECTION BETWEEN THE SIGNIFICANTLY ASSOCIATED LOCUS WITH LIVER TRANSAMINASES

To understand a putative biological connection between the significantly associated locus with levels of liver transaminases, we used a strategy for exploring biomolecular interactions, based on the Cognoscente program, freely available at http://vanburenlab.tamhsc.edu/cognoscente.html. The interaction network image is shown in Figure 2, and comprises 828 nodes with different levels of complexity. Hypernodes, such as the ones centered on *CHUK* and *GOT1*, and nodes-such as the ones centered on *SAMM50*, *MAPK10*, *CD93*, *CELF2* and *BRD7*-are highlighted in Figure 2. The results of the prediction of biomolecular interactions revealed some attractive findings that deserve further exploration in future experimental or functional studies. For example, *GOT1* was predicted to have a significant number of gene-gene interactions, including *IDH1* (isocitrate dehydrogenase 1 (NADP+), soluble) that catalyzes the oxidative decarboxylation of isocitrate to 2-oxoglutarate, *SCD1* (stearoyl-CoA desaturase) involved in fatty acid biosynthesis, which we and others found deregulated in fatty liver[40,41], *GDH2* (glutamate dehydrogenase 2) that catalyzes the reversible oxidative deamination of glutamate to 2-ketoglutarate, and *CHD1* (chromodomain helicase DNA binding protein 1), an ATP-dependent chromatin-remodeling factor that functions as substrate recognition component of the transcription regulatory histone acetylation (HAT) complex SAGA, (Figure 3A, arrow).

Likewise, remarkable gene-gene interactions were noted for *CHUK* and *PPARGC1β* (peroxisome proliferator-activated receptor gamma, coactivator 1 beta), whereby the last one is involved in fat oxidation,

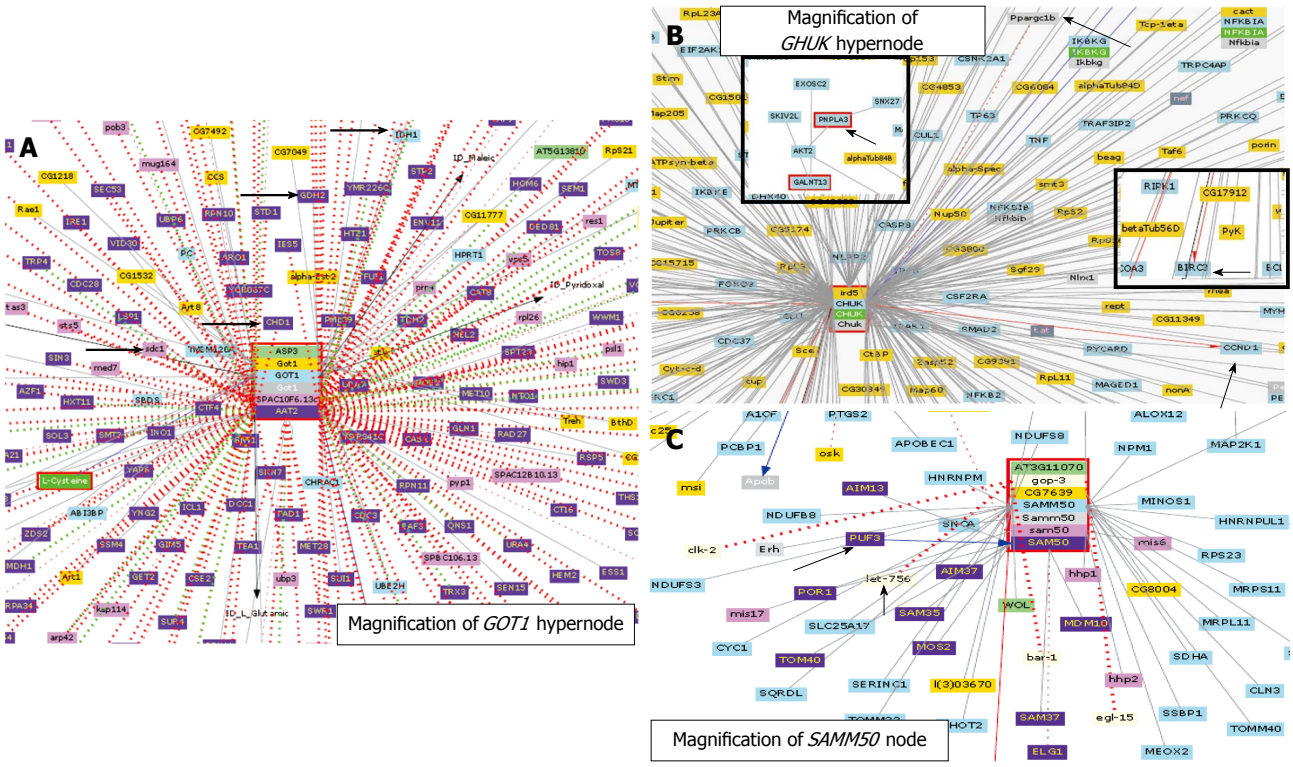

Figure 3 Biomolecular interactions focused on hypernodes (*GOT1* and *CHUK*) and nodes (*SAMM50*) predicted by the visualization tool for systems biology Cognoscente. Cognoscente currently contains over 413000 documented interactions, with coverage across multiple species, including Homo sapiens, Saccharomyces cerevisiae, Drosophila melanogaster, Schizosaccharomyces pombe, Arabidopsis thaliana, Mus musculus, and Caenorhabditis elegans, among others[74]. Colors under the hypernode/node gene name denote different species; for example, light blue corresponds to homo sapiens, blue to saccharomyces cerevisiae S288c and violet to schizosaccharomyces pombe; light green is arabidopsis thaliana, orange is Drosophila melanogaster, red is Gallus gallus, gray is rattus norvegicus and pale gray is caenorhabditis elegans. Arrows highlight biomolecular interactions discussed in the body of the manuscript.

non-oxidative glucose metabolism, and the regulation of energy expenditure (Figure 3B, arrow). Gene-protein interactions between *CHUK* and CCND1 (cyclin D1), a highly conserved member of the cyclin family (Figure 3B, arrow), and BIRC3 (baculoviral IAP repeat containing 3) (Figure 3B, inset), a gene activated by hypoxia we previously found associated with NAFLD in a human study[42], were predicted. PNPLA3 showed a connection with the CHUK hypernode (Figure 3B, inset) by a putative protein-protein interaction with AKT2 (v-akt murine thymoma viral oncogene homolog 2), which regulates many processes, including metabolism, proliferation, cell survival, growth and angiogenesis.

Systems Biology modeling also predicted presence of gene-gene biomolecular interaction between *SAMM50* and let-756 (Figure 3C, arrow), whereby the latter is involved in fibroblast growth factor receptor signaling pathway in Caenorhabditis elegans. In addition, a protein-RNA prediction between SAMM50 and PUF3 was found (Figure 3C, arrow); PUF proteins bind to related sequence motifs in the 3' untranslated region of specific target mRNAs and repress their translation[43].

GWAS COUPLED WITH METABOLOMICS ANALYSIS: UNDERSTANDING THE GENETIC CONTRIBUTIONS TO METABOLIC DIVERSITY

The use of GWAS studies, coupled with large scale metabolomics analysis, is a powerful strategy that can assist in better understanding genetic contributions to metabolic diversity and its importance in the biological context. For instance, Geiger and colleagues performed a GWAS with metabolomics based on the quantitative measurement of 363 metabolites in serum, and found that common variants might explain up to 12% of the observed variance in metabolite concentration[44]. Examples of GWAS relevance are the discovery of SNPs in *GLS2* (glutamine synthase 2) associated with glutamine levels, such as the rs2638315[45]. Furthermore, a recently reported large GWAS coupled with high-throughput metabolomics demonstrated the role of genetic loci in influencing human metabolism, including liver enzymes[46]. Shin *et al*[46] explored genome-wide

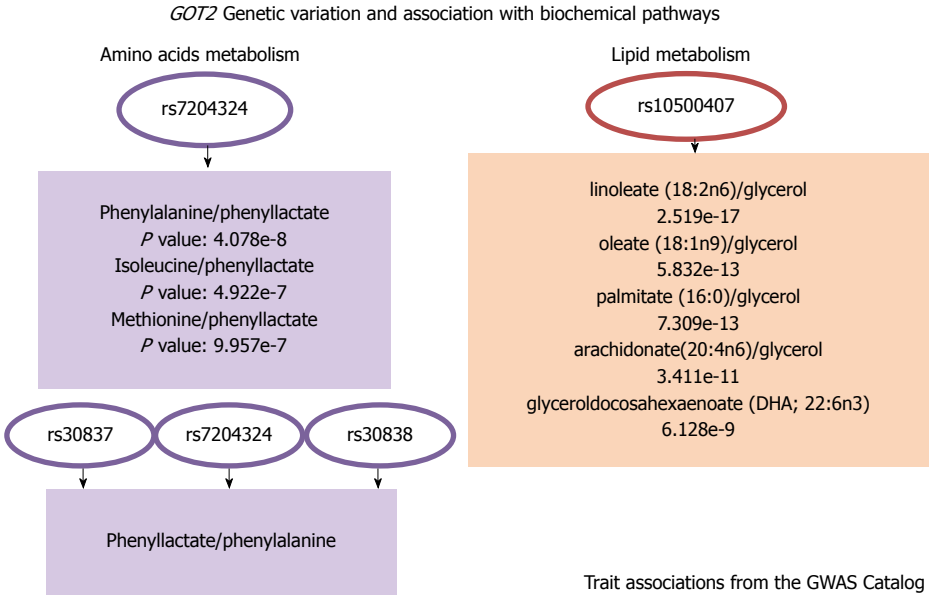

Figure 4 *GOT2*: Trait associations from the genome-wide association studies Catalog. Significant associations were extracted from the Metabolomics genome-wide association studies Server, freely available at http://metabolomics.helmholtz-muenchen.de/gwas/index.php. This site contains the association results of two genome-wide association studies on the human metabolome[46,78]. GWAS: Genome-wide association studies.

associations at 145 metabolic loci and their biochemical connectivity with more than 400 metabolites in human blood and found that a variant in *GOT2,* the rs12709013, was associated with the phenyllactate/phenylalanine ratio. In addition, the *GOT2*-rs4784054 was associated with phenyllactate levels[46]. Interestingly, the rs12709013 and the rs4784054, located in chromosome 16, at positions 58795886 and 58742410, respectively (both in the forward strand), are intronic variants that reside in a genomic region encoding a noncoding RNA (ncRNA) transcript (Gene: RNU6-1155P ENSG00000200424). Genetic regulation of these metabolites may indicate that *GOT2* would be not only involved in the maintenance of the equilibrium of amino acid levels in circulation, including the regulation of the glucose-alanine cycle, but also in the control of energy balance. Figure 4 depicts additional variants in *GOT2* with genome-wide significant associations with metabolites of the amino acid and lipid metabolism. Surprisingly, these findings indicate that the metabolites associated with *GOT2* locus surpass the current knowledge of classical GOT2 protein function. They thus offer novel biochemical and functional insights into poorly explored roles of GOT2, such as fatty acid and glycerolipid metabolism. For instance, a significant association between the intronic *GOT2*-rs10500407 and arachidonate (20:4n6)/glycerol ratio was recently reported[46] (Figure 4), suggesting that GOT2 participates in the arachidonate and docosahexaenoic acid (DHA)-related metabolic pathways. Likewise, variants located in *GOT1* (rs11867, *P* value < 0.0008 and rs10748775, *P* value < 0.001) were associated with N-(2-h) glycine and glycerol 2-phosphate, respectively.

Overall, these observations are in line with the functional data from comparative genomics, indicating that the domain and molecular function of aminotransferases, including GOT2, are highly conserved among species, thus suggesting an important role in metabolic functioning. For instance, the orthologue of *GOT2* in drosophila melanogaster, known as Dmel/Got2, is involved in glutamate[47] and aspartate metabolic process[48]. Moreover, tissue localization yielded evidence, indicating that Dmel\Got2 is expressed in embryonic larval fat body and midgut[49], and in adult heart[50]. Furthermore, subcellular localization showed evidence of Dmel\Got2 localized to lipid particles[51] and mitochondria[52]. Notably, drosophila lipid droplets are ubiquitous organelles, which play a central role in cholesterol homeostasis and lipid metabolism. Taken together, these observations suggest that metabolism regulation is the "ancestral" GOT2 protein function. A representative figure of *GOT2* gene phylogenetic tree is depicted in Figure 5, where, supporting the aforementioned concept, the high conservation of the aminotransferase I and II domains among species, including fruit fly, is clearly visible.

On the other hand, variants located in *GPT1* were associated with epiandrosterone sulfate and androsterone sulfate (rs1063739, *P* value < 0.0002 and 0.0007, respectively), and variants located in *GPT2* were associated with 3-phenylpropionate (hydrocinnamate) (rs734309, *P* value < 0.00001) and the ratio between biliverdin and glycoursodeoxycholate (rs754043, *P* value < 1×10^{-7}).

Finally, metabolite associations with *PNPLA3*-rs738409 are summarized in Table 5. Notably, while no significant associations were found for the rs738409 and human metabolites, some other SNPs in disequilibrium with this variant reached GWAS significance with metabolite ratios of interest, for example cholesterol/gamma-glutamyltyrosine, docosapentaenoate (n3 DPA; 22:5n3)/eicosapentaenoate (EPA; 20:5n3), and aspartylphenylalanine/docosapentaenoate (n3 DPA; 22:5n3) (Table 5). Remarkably,

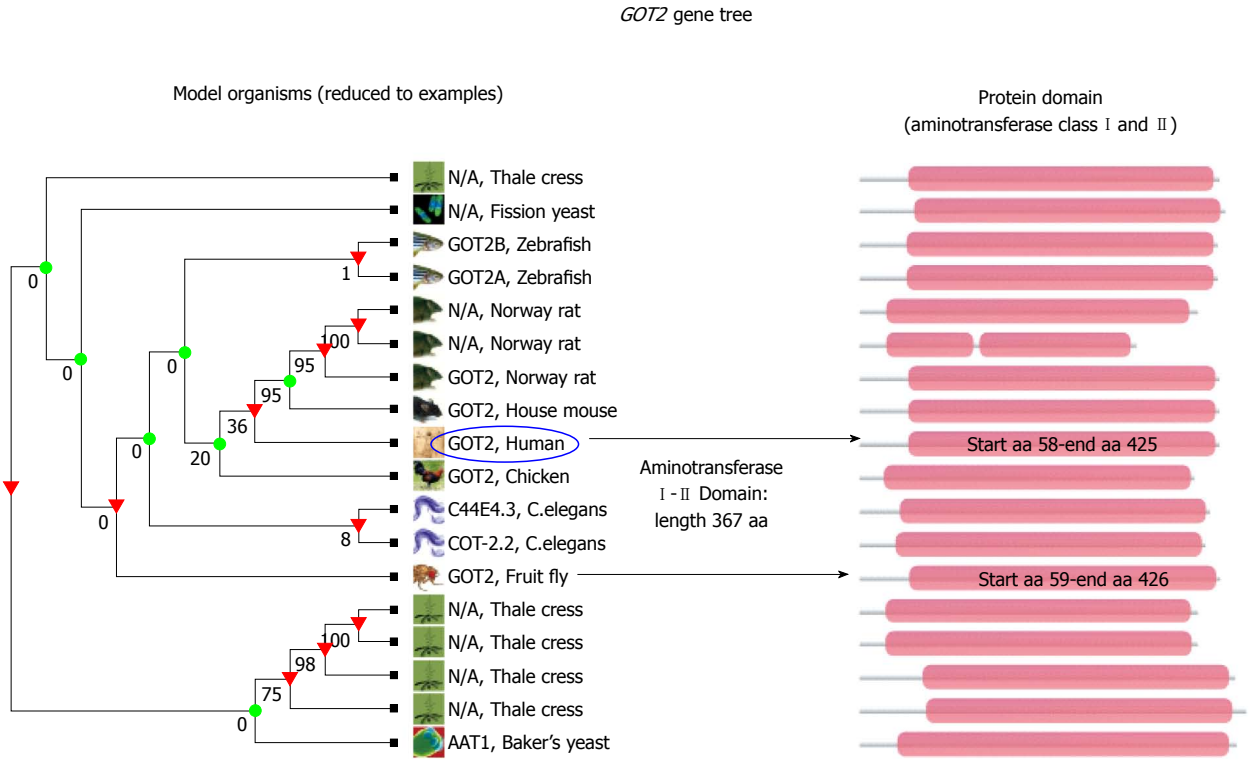

Figure 5 Conservation analysis of *GOT2* between species. Cladogram shows the relationships between *GOT2* genes in different species; for simplicity reasons species were restricted to few models. An alignment of all homologous sequences (protein domain) in the TreeFam family is represented in pink, displayed on the left side of the graph. Numbers below branches are bootstrap values, whereby 100% indicates strong support for these nodes, whereas other nodes receive much weaker support (*e.g.*, 0%). Arrows highlight aminotransferase class Ⅰ and Ⅱ domain in human and fruit fly (aa: amino acid). TreeFam gene was created by using the resource TreeFam, freely available at http://www.treefam.org/family.

eicosapentaenoic acid (EPA) is an important polyunsaturated fatty acid that serves as a precursor for the prostaglandin-3 and thromboxane-3 families. In our previous works, putative disease-related mechanisms associated with PNPLA3 severity in NASH and its relationship with prostaglandins were postulated[53,54]. Furthermore, aspartylphenylalanine results from an incomplete breakdown of protein digestion products, or protein catabolism, and might have a physiological involvement in cell-signaling. Recently, we described a novel role of PNPLA3, beyond its classical participation in triacylglycerol remodeling that involves amino acid metabolism[55]. Furthermore, the importance of amino acid metabolism in the pathogenesis of NAFLD was recently highlighted[56,57]. Finally, a significant association was found between the intronic *PNPLA3*-rs2281135 and the glycocholate (glycocholic acid) to levulinate (4-oxovalerate) ratio. The secondary bile glycocholic acid is a bile acid-glycine conjugate produced by the action of enzymes existing in the microbial flora of the colonic environment. Metabolomic data on *PNPLA3* also indicated that this gene plays an important role in bile acid metabolism[55].

A comprehensive summary of SNPs and their role in the regulation of metabolites can be found at the freely available web resource "Metabolomics GWAS server" (http://metabolomics.helmholtz-muenchen.de/)[46].

CONCLUSION

The reaction of transamination from glutamine to alpha-keto acids was first described in 1950[4]. Since then, clinicians have been using serum measurement of ALT and AST for the evaluation of liver injury. Nearly 50 years later, the rise in the worldwide prevalence of obesity, type 2 diabetes and CVD brought into the clinical scenario a new concept, associating increased levels of liver enzymes with long-term development of multiple metabolic and CV disorders. In addition, GWAS coupled with metabolomics uncovered key roles of transaminases in the global metabolism. In particular, "omics" studies have led to interesting insights into the biology of liver metabolic function and its relationship with liver transaminases.

Of note, background knowledge on liver enzymes functioning indicates that liver concentrations of ALT and AST are not significantly higher relative to the pool of major liver enzymes[58]. In contrast, liver concentrations of LDH (lactate dehydrogenase) and MD (malate dehydrogenase), for instance, rank first and second, respectively. At 50% of their concentrations, AST and ALT respectively take the third and fourth place (Figure 6)[58].

Wieme *et al*[58] made an interesting observation about the relationship between liver and plasma concentration of

Table 5 *PNPLA3*-rs738409 metabolite trait associations from the genome-wide association studies catalog

Variant ID	LD with rs738409	Metabolite ratio	*P* value for association
rs12483959	0.657	Cholesterol/gamma-glutamyltyrosine	7.76×10^{-6}
rs2076211	0.657	Cholesterol/gamma-glutamyltyrosine	1.19×10^{-5}
rs2076211	0.657	Aspartylphenylalanine/docosapentaenoate (n3 DPA; 22:5n3)	5.26×10^{-6}
rs2294922	0.657	Docosapentaenoate (n3 DPA; 22:5n3)/eicosapentaenoate (EPA; 20:5n3)	4.64×10^{-7}
rs2073081	0.568	3-methoxytyrosine/gamma-glutamylthreonine	2.14×10^{-5}
rs2281135	0.609	Glycocholate/levulinate (4-oxovalerate)	3.26×10^{-5}
rs1010023	0.609	Docosapentaenoate (n3 DPA; 22:5n3)/phenylacetylglutamine	1.43×10^{-5}
rs926633	0.609	Docosapentaenoate (n3 DPA; 22:5n3)/myristate (14:0)	1.08×10^{-5}
rs2896019	0.607	Aspartylphenylalanine/docosapentaenoate (n3 DPA; 22:5n3)	1.31×10^{-5}

Information was retrieved from the GWAS server at the freely accessible URL: http://metabolomics.helmholtz-muenchen.de. This server combines data on large GWAS and non-targeted and metabolome-wide panel of small molecules, using blood samples and phenotype data from 2824 individuals of two major population-based European cohorts: the German KORA study and the British TWINs UK study. LD: Linkage disequilibrium that refers to a non-random association in the occurrence of alleles at two loci was assessed by R^2 (the square of the correlation coefficient between the presence or absence of a particular allele at the first locus and the other representing the presence or absence of a particular allele at the second locus). GWAS: Genome-wide association studies.

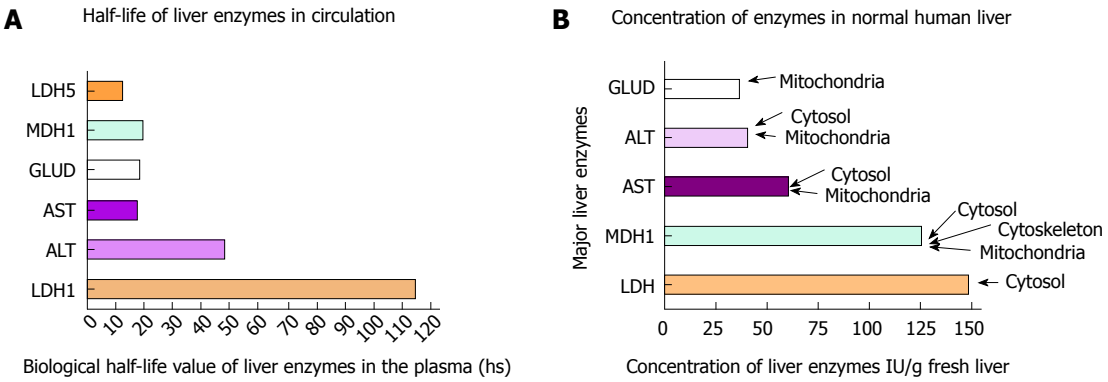

Figure 6 Liver enzyme concentrations in normal human liver. Information of the liver concentration of major liver enzymes was extracted from the report published by Wieme *et al*[58]. Evidence of subcellular locations from the Compartments database (http://compartments.jensenlab.org) is highlighted. LDH5: Human isoform-5 of lactate dehydrogenase, which is normally present in the liver; GLUD: Glutamate dehydrogenase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; MDH1: Malate dehydrogenase; LDH: Lactate deydrogenase.

enzymes, reporting that the localization of the enzymes at the cellular level very much conditions the concentration in the circulation. The authors further showed that mild cell damage tends to release the enzymes in the soluble fraction only, while severe necrotic lesions, which also affect the mitochondria, release enzymes from both fractions[58]. Hence, it is plausible to suggest that enzymes that are present in high concentrations in the liver tissue, such as LDH, might better and more accurately reflect liver injury. Nevertheless, LDH levels are not commonly used in clinical practice for the diagnosis or monitoring of acute or chronic liver damage. Indeed, serum LDH was reported to be markedly elevated in ischemic, but not viral hepatitis[59,60], indicating that severe cell necrosis is needed for the leaking of the liver-LDH content into the blood to occur.

A second factor in determining the rise of liver enzymes in the circulation seems to be its biological half-life as, according to the observation of Wieme *et al*[58], the longer the half-life the greater the accumulation of the enzymes in the serum (Figure 6 depicts the half-life of liver enzymes in serum). Based on the above, the findings reported by Wieme *et al*[58] may explain why ALT, which seems to have a half-life of 50 h, is more likely to be found elevated in serum than the LDH isoform 5, which is normally present in the liver and has a very short half-life of 10 h.

Taken together, the observations made in the GWAS studies, coupled with metabolomics, in conjunction with past knowledge on the traditional biochemistry explorations (presently referred to as "quantitative biology"), are of particular significance in providing a plausible biological explanation to the meaning of elevated ALT and AST levels in the clinical setting. Indeed, these findings suggest that restricting the biological role of elevated aminotransferase levels to liver injury has not only been a misinterpretation but an underestimation of the biological role of these enzymes. Interestingly, *in vitro* studies showed that even ethanol might increase transaminases, mitochondrial AST in particular, by up-regulating gene expression, rather than by inducing its release owing to cell injury[61].

Data from the Third National Health and Nutrition Examination Survey, the largest epidemiological study in the United States, showed that the prevalence of aminotransferase elevation in the general population is

about 9%[22]. Notably, unexplained enzyme elevations were associated with adiposity and other features of the metabolic syndrome[22,62]. On the other hand, presence of a considerable inter-individual variation in the level of transaminases is widely acknowledged, and this variability is explained in part by genetic variation. Links between aminotransferase genes and metabolites demonstrated important contributions to the metabolic diversity. In fact, evidence from high-throughput studies of genetic influences on human metabolites demonstrated that aminotransferases ALT and AST are not solely involved in gluconeogenesis and amino acid synthesis, but also regulate other functions of the liver metabolism, such as fatty acid, glycerolipid and bile acids metabolism. In addition, an interesting study highlighted a putative relationship between iron stores, ALT activity and the risk of metabolic disturbances in adolescents that deserves further follow-up[63].

Finally, the role of the newly described TM6SF2 rs58542926 nonsynonymous variant in genetic susceptibility to NAFLD and disease severity[64] deserves follow-up as this variant was associated in population-based studies but not in NAFLD patients with levels of transaminases in circulation[65].

In conclusion, genomic, transcriptomic, proteomic, and metabolomic information has changed the classical conception of the meaning that serum concentrations of ALT and ALT are merely indicators of hepatocyte membrane disruption. It has given way to a more complex and interconnected view of the importance of liver transaminases in the regulation of systemic metabolic function.

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