

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

Silvia Sookoian, Carlos J Pirola

Silvia Sookoian, Department of Clinical and Molecular Hepatology, Institute of Medical Research A Lanari-IDIM, University of Buenos Aires - National Scientific and Technical Research Council Ciudad Autónoma de Buenos Aires, Buenos Aires 1427, Argentina

Carlos J Pirola, Department of Molecular Genetics and Biology of Complex Diseases, Institute of Medical Research A Lanari-IDIM, University of Buenos Aires - National Scientific and Technical Research Council, Ciudad Autónoma de Buenos Aires, Buenos Aires 1427, Argentina

Author contributions: Pirola CJ and Sookoian S contributed to study concept and design; acquisition of data; analysis and interpretation of data; statistical analysis; drafting of the manuscript; obtained funding and study supervision.

Supported by (in part) Grants PICT 2010-0441 and PICT 2012-0159 (Agencia Nacional de Promoción Científica y Tecnológica) and UBACYT CM04 (Universidad de Buenos Aires).

Conflict-of-interest: The authors have no conflict of interest to declare.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Silvia Sookoian, MD, PhD, Department of Clinical and Molecular Hepatology, Institute of Medical Research A Lanari-IDIM, University of Buenos Aires - National Scientific and Technical Research Council, Ciudad Autónoma de Buenos Aires, Combatiente de Malvinas 3150, Buenos Aires 1427, Argentina. sookoian.silvia@lanari.fmed.uba.ar

Telephone: +54-11-45148701

Fax: +54-11-45238947

Received: September 4, 2014

Peer-review started: September 4, 2014

First decision: October 14, 2014

Revised: October 18, 2014

Accepted: December 14, 2014

Article in press: December 16, 2014

Published online: January 21, 2015

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

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

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.

Sookoian S, Pirola CJ. Liver enzymes, metabolomics and genome-wide association studies: From systems biology to the personalized medicine. *World J Gastroenterol* 2015; 21(3): 711-725 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i3/711.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i3.711>

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 aminotransferase 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 reliable 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 formally *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

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

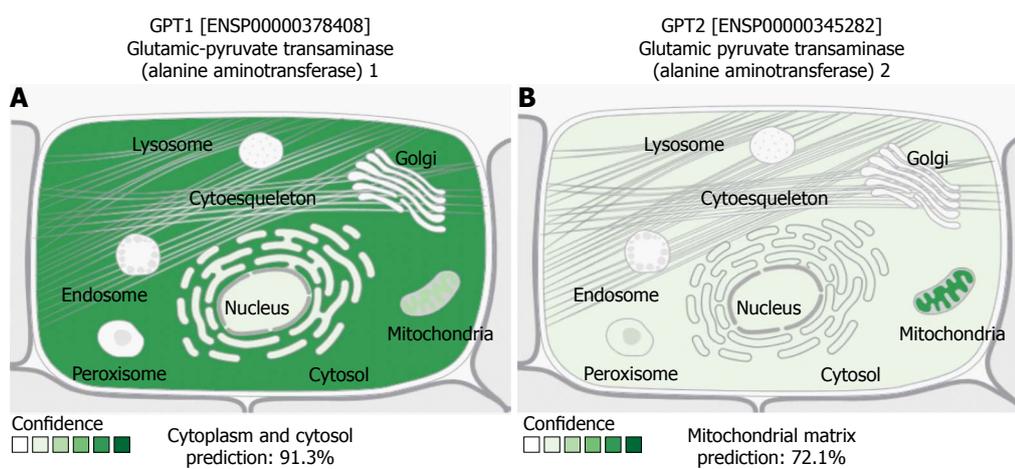


Figure 1 Schematic representation of different localizations of ALT1 (GPT1) and ALT2 (GPT2) proteins at the cellular level. Prediction was performed by the open access web resource "COMPARTMENTS" available at <http://compartments.jensenlab.org>, which predicts protein localization according to information 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 %.

mitochondria^[18]. Conversely, ALT2 is preferably localized in the mitochondrial matrix (Figure 1). Thus, while current 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

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) <i>GPT1</i>	Glutamic pyruvate transaminase 2 (alanine aminotransferase) 2 <i>GPT2</i>
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	<i>GPT1</i> has 210 SNPs	<i>GPT2</i> has 819 SNPs
Orthologues	<i>GPT1</i> has 69 orthologues in Ensembl	<i>GPT2</i> has 63 orthologues in Ensembl
Regulation	There are 2 regulatory elements located in the region of <i>GPT1</i> gene miR-122 may interact with <i>GPT1</i> 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] <i>GPT1</i> , but not <i>GPT2</i> 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 <i>GPT2</i> <i>GPT2</i> promoter has a putative ATF4 (Activating transcription factor 4 binding site) ^[69] <i>GPT2</i> 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] 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]
Tissue expression in rodents	Evidence: NB (mRNA): Highly expressed in liver and moderately expressed in white adipose tissue (WAT), intestine, and colon ^[71]	Gene expression analysis suggests a sex-dependent difference in <i>GPT2</i> -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 (<i>ob/ob</i>): Compared to the normal liver of lean mice, the expression of <i>GPT1</i> 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 (<i>ob/ob</i>): Compared to the normal liver of lean mice, the expression of <i>GPT2</i> mRNA was elevated by about 2-fold, suggesting ALT2 induction during fatty liver ^[71]

¹Cellular 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 <i>et al</i> ^[36]	<i>n</i> = 61089 Population-based Adults	Affymetrix, Illumina and perlegen sciences arrays	About 2.6 million directly genotyped or imputed autosomal SNPs	Plasma levels of ALT	<i>HSD17B13, MAPK10, TRIB1, CPN1, PNPLA3, SAMM50</i>
Yuan <i>et al</i> ^[74]	Initial study <i>n</i> = 7715 Replication <i>n</i> = 704 Population-based Adults	Affymetrix	-	Plasma levels of ALT	<i>CHUK, PNPLA3, SAMM50, CPN1</i>
Park <i>et al</i> ^[75]	<i>n</i> = 532 Population-based Children	Illumina HumanOmni1-Quad BeadChip	747076 SNPs	Plasma levels of ALT	<i>ST6GALNAC3, MMADHC, CCDC102B, RGS5, BRD7, GALNT13, SIRPA, CD93, SLC39A11, ADAMTS9, CELF2</i>
Shen <i>et al</i> ^[76]	<i>n</i> = 866 Population-based adults	Affymetrix GeneChip Human Mapping 500 K Array set	500568 SNPs	Plasma levels of AST	<i>CYB5APS, CELF2, GOT1, ST6GALNAC3, ADAMTS9, THSD7B, EIF4A1P1, ROBO1, THSD7B GOT1</i>

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

development and in adipocyte 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 aminotransferase levels, their main features, and the biological role of the gene where are they located is shown in Table 4.

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}	<i>HSD17B13/</i> <i>MAPK10</i>	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}	<i>TRIB1</i>	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}	<i>CPN1</i>	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	1.2×10^{-45}	<i>PNPLA3</i>	Acylglycerol O-acyltransferase and triacylglycerol lipase that mediates triacylglycerol hydrolysis
	p.Ile148Met			
rs2281135	Intron variant	8.2×10^{-12}		
rs3761472	Missense	3.7×10^{-29}	<i>SAMM50</i>	Component of the sorting and assembly machinery (SAM) of the mitochondrial outer membrane
	p.Asp110Gly			
rs2143571	Intron variant	9.4×10^{-7}		
rs11597086	Non coding exon variant	3.6×10^{-7}	<i>CHUK</i>	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}	<i>ST6GALNAC3</i>	Transfer sialic acids from CMP-sialic acid to terminal positions of carbohydrate groups in glycoproteins and glycolipids
rs17801127	Intergenic	2.37×10^{-7}	<i>MMADHC</i>	Mitochondrial protein that is involved in an early step of vitamin B12 metabolism
rs1539893	Intron variant	3.40×10^{-6}	<i>CCDC102B</i>	Unknown
rs12035879	Intron variant	3.97×10^{-6}	<i>RGS5</i>	Member of the regulators of G protein signaling (RGS) family
rs9941219	Intergenic	4.06×10^{-6}	<i>BRD7</i>	Member of the bromodomain-containing protein family
rs731660	Intergenic			
rs12621256	Intron variant	4.36×10^{-6}	<i>GALNT13</i>	Member of the glycosyltransferase 2 family; catalyzes the initial reaction in oligosaccharide biosynthesis; neurons cell biogenesis
rs6035126	Intergenic	4.94×10^{-6}	<i>SIRPA</i>	Receptor-type transmembrane glycoproteins involved in the negative regulation of receptor tyrosine kinase-coupled signaling processes
rs13433286	Intergenic			
rs844917	Intergenic	5.64×10^{-6}	<i>CD93</i>	Cell-surface glycoprotein and type I membrane protein
rs844914	Intergenic	5.98×10^{-6}		
rs903107	Intron variant	6.11×10^{-6}	<i>SLC39A11</i>	Mediates zinc uptake
rs80311637	Missense	7.18×10^{-6}	<i>ADAMTS9</i>	Disintegrin and metalloproteinase with thrombospondin motifs
	p.Val653Met			
rs596406	Intron variant	9.18×10^{-6}	<i>CEL2</i>	RNA-binding protein implicated in the regulation of several post-transcriptional events
AST				
rs11597390	Intergenic	0.0009	<i>CHUK</i>	Explained previously
rs2281135	Intron variant	5.7×10^{-6}	<i>PNPLA3</i>	Explained previously
rs862946	Intergenic	2.41×10^{-7}	<i>CYB5AP5</i>	Pseudogene
rs596406	Intron variant	3.69×10^{-7}	<i>CEL2</i>	Explained previously
rs76850691	Missense	8.55×10^{-7}	<i>GOT1</i>	Biosynthesis of L-glutamate from L-aspartate or L-cysteine
	p.Gln349Glu			
rs17109512	Intergenic	2.80×10^{-14}		
rs4949718	Intron variant	1.49×10^{-6}	<i>ST6GALNAC3</i>	Explained previously
rs80311637	Missense	1.85×10^{-6}	<i>ADAMTS9</i>	Explained previously
	p.Val1597Met			
rs892877	Intron variant	3.75×10^{-6}	<i>THSD7B</i>	Unknown
rs984295	Intron variant	5.86×10^{-6}		
rs457603	Intergenic	4.57×10^{-6}	<i>EIF4A1P1</i>	Pseudogene
rs452621	Intergenic			
rs7617400	Intron variant	6.16×10^{-6}	<i>ROBO1</i>	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-acetylneuraminyl-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 metalloproteinase with thrombospondin type 1 motif, 9; *CYB5AP5*: Cytochrome b5 type A (microsomal) pseudogene 5; *CEL2*: CUGBP, Elav-like family member 2; *GOT1*: Glutamic-oxaloacetic transaminase 1, soluble; *THSD7B*: thrombospondin, type 1, 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.medicine.univ-paris5.fr>).

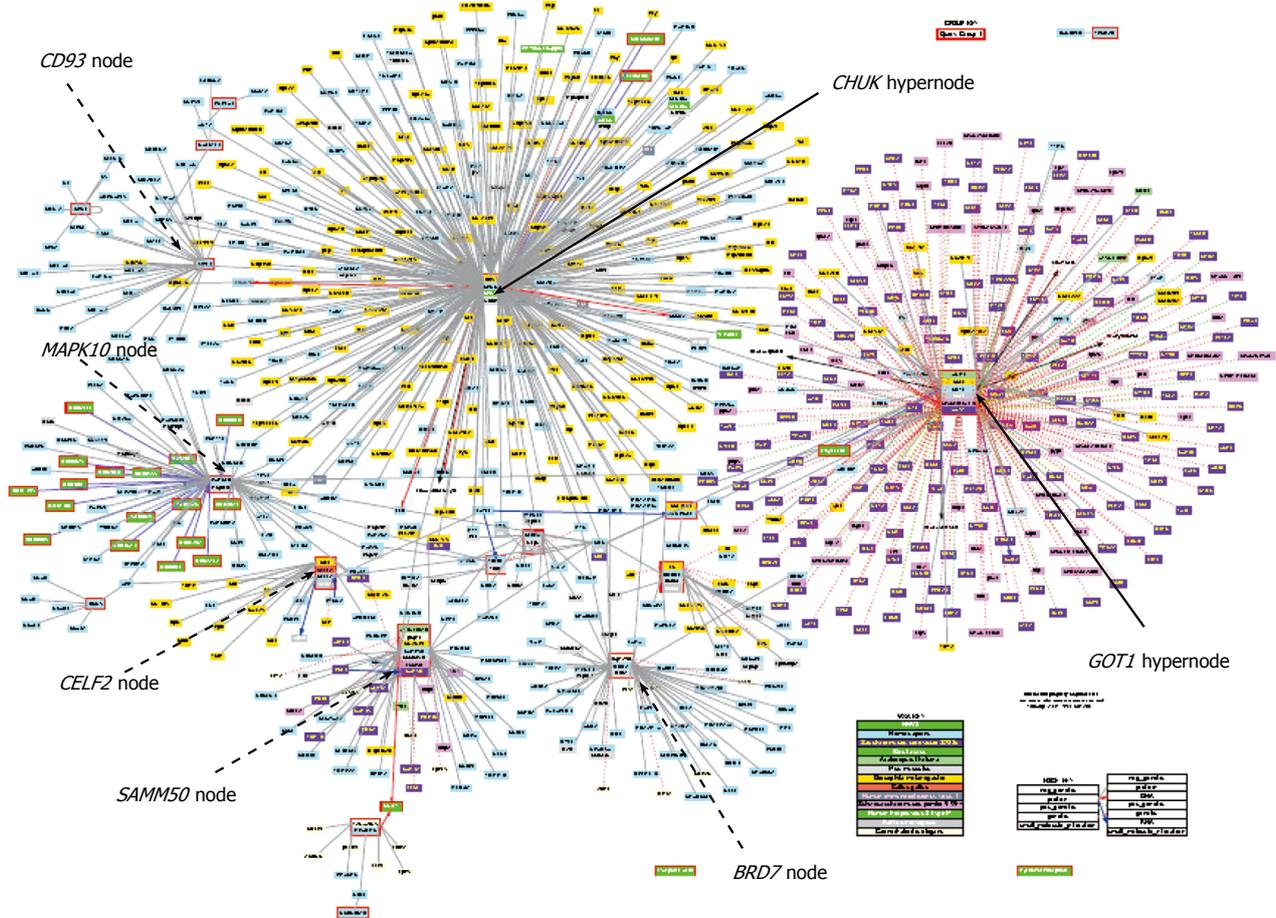


Figure 2 Visualization of biomolecular interactions among associated loci with serum levels of alanine-aminotransferase and aspartate-aminotransferase in published genome-wide association studies. Prediction was based on the Cognoscente program, freely available at the web-based submission portal: <http://vanburenlab.tamhsc.edu/cognoscente.html>. 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.

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 *PPARGC1B* (peroxisome proliferator-activated receptor gamma, coactivator 1 beta), whereby the last one is involved in fat oxidation, 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

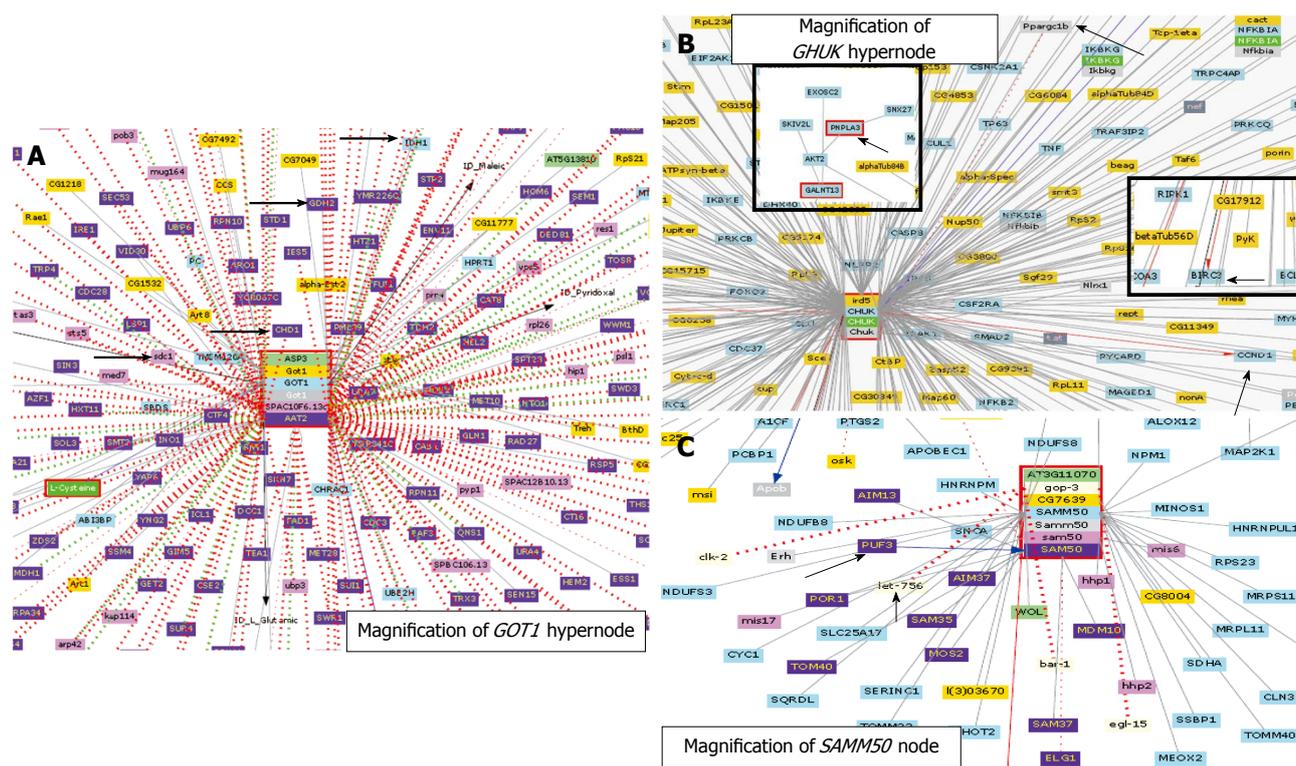


Figure 3 Biomolecular interactions focused on hypernodes (*GOT1* and *CHUK*) and nodes (*SAMM50*) predicted by the visualization tool for systems biology Cognoscente. Cognoscente currently contains over 413000 documented interactions, with coverage across multiple species, including *Homo sapiens*, *Saccharomyces 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.

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 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

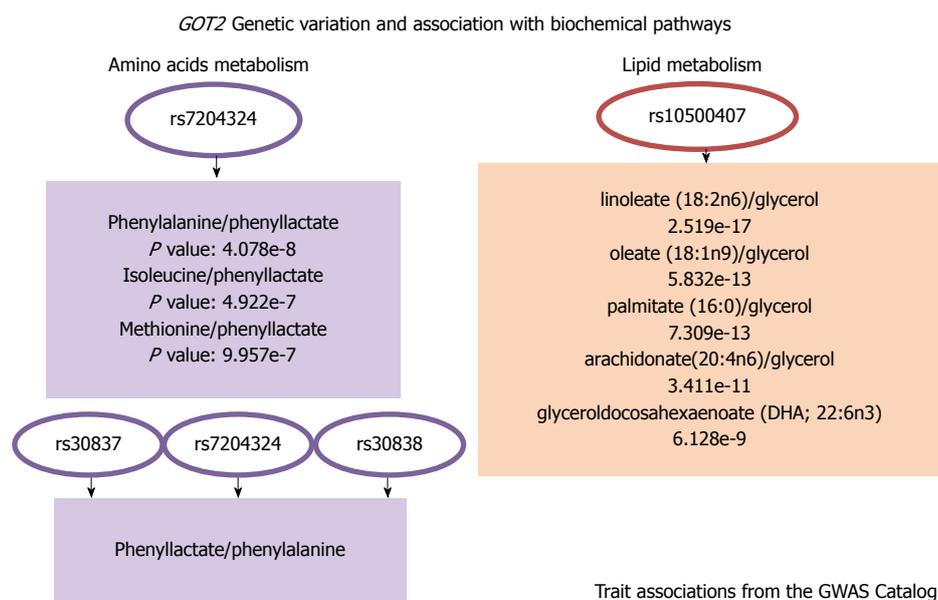


Figure 4 *GOT2*: Trait associations from the genome-wide association studies Catalog. Significant associations were extracted from the Metabolomics genome-wide association studies Server, freely available at <http://metabolomics.helmholtz-muenchen.de/gwas/index.php>. This site contains the association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

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 glycooursodeoxycholate (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, 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

GOT2 gene tree

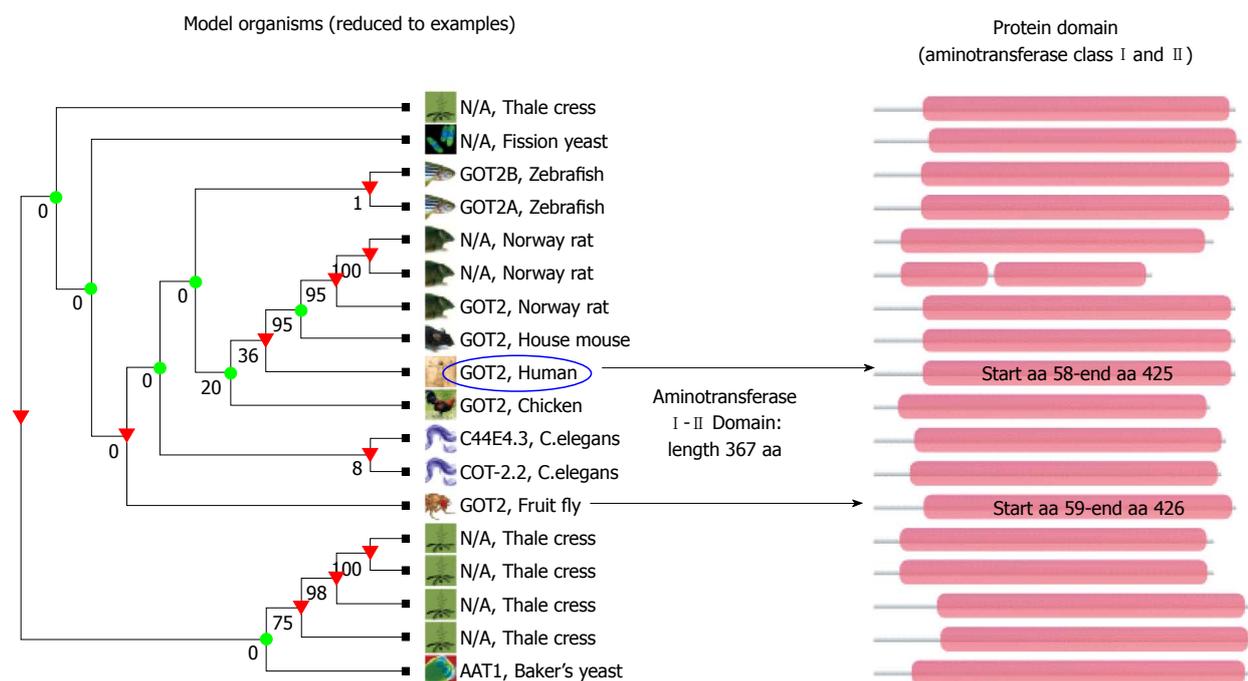


Figure 5 Conservation analysis of GOT2 between species. Cladogram shows the relationships between GOT2 genes in different species; for simplicity reasons species were restricted to few models. An alignment of all homologous sequences (protein domain) in the TreeFam family is represented in pink, displayed 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>.

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 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.

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.

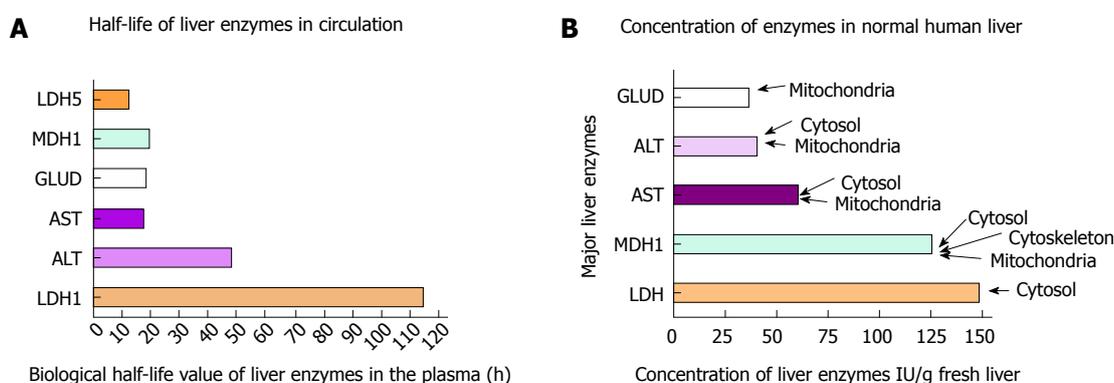


Figure 6 Liver enzyme concentrations in normal human liver. Information of the liver concentration of major liver enzymes was extracted from the report published by Wieme *et al.*^[58]. Evidence of subcellular locations from the Compartments database (<http://compartments.jensenlab.org>) is highlighted. 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 dehydrogenase.

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.

REFERENCES

- 1 **Pratt DS**, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N Engl J Med* 2000; **342**: 1266-1271 [PMID: 10781624 DOI: 10.1056/NEJM200004273421707]
- 2 **Molander DW**, Wroblewski F, Ladue JS. Serum glutamic oxalacetic transaminase as an index of hepatocellular integrity. *J Lab Clin Med* 1955; **46**: 831-839 [PMID: 13271882]
- 3 **Killip T**, Payne MA. High serum transaminase activity in heart disease. Circulatory failure and hepatic necrosis. *Circulation* 1960; **21**: 646-660 [PMID: 14409072]
- 4 **Meister A**, Tice SV. Transamination from glutamine to alpha-keto acids. *J Biol Chem* 1950; **187**: 173-187 [PMID: 14794702]
- 5 **Sherman KE**. Alanine aminotransferase in clinical practice. A review. *Arch Intern Med* 1991; **151**: 260-265 [PMID: 1992953]
- 6 **Kamiike W**, Fujikawa M, Koseki M, Sumimura J, Miyata M, Kawashima Y, Wada H, Tagawa K. Different patterns of leakage of cytosolic and mitochondrial enzymes. *Clin Chim Acta* 1989; **185**: 265-270 [PMID: 2620458]
- 7 **Reichling JJ**, Kaplan MM. Clinical use of serum enzymes in liver disease. *Dig Dis Sci* 1988; **33**: 1601-1614 [PMID: 2904353]
- 8 **Sattar N**, Scherbakova O, Ford I, O'Reilly DS, Stanley A, Forrest E, Macfarlane PW, Packard CJ, Cobbe SM, Shepherd J. Elevated alanine aminotransferase predicts new-onset type 2 diabetes independently of classical risk factors, metabolic syndrome, and C-reactive protein in the west of Scotland coronary prevention study. *Diabetes* 2004; **53**: 2855-2860 [PMID: 15504965]
- 9 **Vozarova B**, Stefan N, Lindsay RS, Saremi A, Pratley RE, Bogardus C, Tataranni PA. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 2002; **51**: 1889-1895 [PMID: 12031978]
- 10 **Ioannou GN**, Weiss NS, Boyko EJ, Mozaffarian D, Lee SP. Elevated serum alanine aminotransferase activity and calculated risk of coronary heart disease in the United States. *Hepatology* 2006; **43**: 1145-1151 [PMID: 16628637 DOI: 10.1002/hep.21171]
- 11 **Kain K**, Carter AM, Grant PJ, Scott EM. Alanine aminotransferase is associated with atherothrombotic risk factors in a British South Asian population. *J Thromb Haemost* 2008; **6**: 737-741 [PMID: 18315552 DOI: 10.1111/j.1538-7836.2008.02935.x]
- 12 **Song HR**, Yun KE, Park HS. Relation between alanine aminotransferase concentrations and visceral fat accumulation among nondiabetic overweight Korean women. *Am J Clin Nutr* 2008; **88**: 16-21 [PMID: 18614719]
- 13 **Goessling W**, Massaro JM, Vasan RS, D'Agostino RB, Ellison RC, Fox CS. Aminotransferase levels and 20-year risk of metabolic syndrome, diabetes, and cardiovascular disease. *Gastroenterology* 2008; **135**: 1935-1944, 1944.e1 [PMID: 19010326 DOI: 10.1053/j.gastro.2008.09.018]
- 14 **Sookoian S**, Pirola CJ. Alanine and aspartate aminotransferase and glutamine-cycling pathway: their roles in pathogenesis of metabolic syndrome. *World J Gastroenterol* 2012; **18**: 3775-3781 [PMID: 22876026 DOI: 10.3748/wjg.v18.i29.3775]
- 15 **Yang RZ**, Blaileanu G, Hansen BC, Shuldiner AR, Gong DW. cDNA cloning, genomic structure, chromosomal mapping, and functional expression of a novel human alanine aminotransferase. *Genomics* 2002; **79**: 445-450 [PMID: 11863375 DOI: 10.1006/geno.2002.6722]
- 16 **Uhlen M**, Oksvold P, Fagerberg L, Lundberg E, Jonasson K, Forsberg M, Zwahlen M, Kampf C, Wester K, Hober S, Wernerus H, Björling L, Ponten F. Towards a knowledge-based Human Protein Atlas. *Nat Biotechnol* 2010; **28**: 1248-1250 [PMID: 21139605 DOI: 10.1038/nbt1210-1248]
- 17 **Lindblom P**, Rafter I, Copley C, Andersson U, Hedberg JJ, Berg AL, Samuelsson A, Hellmold H, Cotgreave I, Glinghammar B. Isoforms of alanine aminotransferases in human tissues and serum--differential tissue expression using novel antibodies. *Arch Biochem Biophys* 2007; **466**: 66-77 [PMID: 17826732 DOI: 10.1016/j.abb.2007.07.023]
- 18 **Glinghammar B**, Rafter I, Lindström AK, Hedberg JJ, Andersson HB, Lindblom P, Berg AL, Cotgreave I. Detection of the mitochondrial and catalytically active alanine aminotransferase in human tissues and plasma. *Int J Mol Med* 2009; **23**: 621-631 [PMID: 19360321]
- 19 **Rafter I**, Gråberg T, Kotronen A, Strömmer L, Mattson CM, Kim RW, Ehrenborg E, Andersson HB, Yki-Järvinen H, Schuppe-Koistinen I, Ekblom B, Cotgreave I, Glinghammar B. Isoform-specific alanine aminotransferase measurement can distinguish hepatic from extrahepatic injury in humans. *Int J Mol Med* 2012; **30**: 1241-1249 [PMID: 22922605 DOI: 10.3892/ijmm.2012.1106]
- 20 **Kechagias S**, Ernersson A, Dahlqvist O, Lundberg P, Lindström T, Nystrom FH. Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. *Gut* 2008; **57**: 649-654 [PMID: 18276725 DOI: 10.1136/gut.2007.131797]
- 21 **Pirola CJ**, Fernández Gianotti T, Castaño GO, Mallardi P, San Martino J, Mora Gonzalez Lopez Ledesma M, Flichman D, Mirshahi F, Sanyal AJ, Sookoian S. Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. *Gut* 2014: Epub ahead of print [PMID: 24973316 DOI: 10.1136/gutjnl-2014-306996]
- 22 **Ioannou GN**, Boyko EJ, Lee SP. The prevalence and predictors of elevated serum aminotransferase activity in the United States in 1999-2002. *Am J Gastroenterol* 2006; **101**: 76-82 [PMID: 16405537 DOI: 10.1111/j.1572-0241.2005.00341.x]
- 23 **Statland BE**, Winkel P, Bokelund H. Factors contributing to intra-individual variation of serum constituents. 2. Effects of exercise and diet on variation of serum constituents in healthy subjects. *Clin Chem* 1973; **19**: 1380-1383 [PMID: 4757367]
- 24 **Statland BE**, Winkel P, Bokelund H. Factors contributing to intra-individual variation of serum constituents. 1. Within-day variation of serum constituents in healthy subjects. *Clin Chem* 1973; **19**: 1374-1379 [PMID: 4757366]
- 25 **Rahmioglu N**, Andrew T, Cherkas L, Surdulescu G, Swaminathan R, Spector T, Ahmadi KR. Epidemiology and genetic epidemiology of the liver function test proteins. *PLoS One* 2009; **4**: e4435 [PMID: 19209234 DOI: 10.1371/journal.pone.0004435]
- 26 **Whitfield JB**, Zhu G, Nestler JE, Heath AC, Martin NG. Genetic covariation between serum gamma-glutamyltransferase activity and cardiovascular risk factors. *Clin Chem* 2002; **48**: 1426-1431 [PMID: 12194918]

- 27 **Bathum L**, Petersen HC, Rosholm JU, Hyltoft Petersen P, Vaupel J, Christensen K. Evidence for a substantial genetic influence on biochemical liver function tests: results from a population-based Danish twin study. *Clin Chem* 2001; **47**: 81-87 [PMID: 11148181]
- 28 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]
- 29 **Sookoian S**, Castaño GO, Burgueño AL, Gianotti TF, Rosselli MS, Pirola CJ. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. *J Lipid Res* 2009; **50**: 2111-2116 [PMID: 19738004 DOI: 10.1194/jlr.P900013-JLR200]
- 30 **Sookoian S**, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011; **53**: 1883-1894 [PMID: 21381068 DOI: 10.1002/hep.24283]
- 31 **Sookoian S**, Pirola CJ. PNPLA3, the history of an orphan gene of the potato tuber protein family that found an organ: the liver. *Hepatology* 2014; **59**: 2068-2071 [PMID: 24122882 DOI: 10.1002/hep.26895]
- 32 **Chamorro AJ**, Torres JL, Mirón-Canelo JA, González-Sarmiento R, Laso FJ, Marcos M. Systematic review with meta-analysis: the I148M variant of patatin-like phospholipase domain-containing 3 gene (PNPLA3) is significantly associated with alcoholic liver cirrhosis. *Aliment Pharmacol Ther* 2014; **40**: 571-581 [PMID: 25060292 DOI: 10.1111/apt.12890]
- 33 **Valenti L**, Rumi M, Galmozzi E, Aghemo A, Del Menico B, De Nicola S, Dongiovanni P, Maggioni M, Fracanzani AL, Rametta R, Colombo M, Fargion S. Patatin-like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. *Hepatology* 2011; **53**: 791-799 [PMID: 21319195 DOI: 10.1002/hep.24123]
- 34 **Viganò M**, Valenti L, Lampertico P, Facchetti F, Motta BM, D'Ambrosio R, Romagnoli S, Dongiovanni P, Donati B, Fargion S, Colombo M. Patatin-like phospholipase domain-containing 3 I148M affects liver steatosis in patients with chronic hepatitis B. *Hepatology* 2013; **58**: 1245-1252 [PMID: 23564580 DOI: 10.1002/hep.26445]
- 35 **Trépo E**, Nahon P, Bontempi G, Valenti L, Falletti E, Nischalke HD, Hamza S, Corradini SG, Burza MA, Guyot E, Donati B, Spengler U, Hillon P, Toniutto P, Henrion J, Franchimont D, Devière J, Mathurin P, Moreno C, Romeo S, Deltenre P. Association between the PNPLA3 (rs738409 C > G) variant and hepatocellular carcinoma: Evidence from a meta-analysis of individual participant data. *Hepatology* 2014; **59**: 2170-2177 [PMID: 24114809 DOI: 10.1002/hep.26767]
- 36 **Chambers JC**, Zhang W, Sehmi J, Li X, Wass MN, Van der Harst P, Holm H, Sanna S, Kavousi M, Baumeister SE, Coin LJ, Deng G, Gieger C, Heard-Costa NL, Hottenga JJ, Kühnel B, Kumar V, Lagou V, Liang L, Luan J, Vidal PM, Mateo Leach I, O'Reilly PF, Peden JF, Rahmioglu N, Soininen P, Speliotes EK, Yuan X, Thorleifsson G, Alizadeh BZ, Atwood LD, Borecki IB, Brown MJ, Charoen P, Cucca F, Das D, de Geus EJ, Dixon AL, Döring A, Ehret G, Eyjolfsson GI, Farrall M, Forouhi NG, Friedrich N, Goessling W, Gudbjartsson DF, Harris TB, Hartikainen AL, Heath S, Hirschfield GM, Hofman A, Homuth G, Hyppönen E, Janssen HL, Johnson T, Kangas AJ, Kema IP, Kühn JP, Lai S, Lathrop M, Lerch MM, Li Y, Liang TJ, Lin JP, Loos RJ, Martin NG, Moffatt MF, Montgomery GW, Munroe PB, Musunuru K, Nakamura Y, O'Donnell CJ, Olafsson I, Penninx BW, Pouta A, Prins BP, Prokopenko I, Puls R, Ruokonen A, Savolainen MJ, Schlessinger D, Schouten JN, Seedorf U, Sen-Chowdhry S, Siminovitch KA, Smit JH, Spector TD, Tan W, Teslovich TM, Tukiainen T, Uitterlinden AG, Van der Klauw MM, Vasani RS, Wallace C, Wallaschofski H, Wichmann HE, Willemsen G, Würtz P, Xu C, Yerges-Armstrong LM, Abecasis GR, Ahmadi KR, Boomsma DI, Caulfield M, Cookson WO, van Duijn CM, Froguel P, Matsuda K, McCarthy MI, Meisinger C, Mooser V, Pietiläinen KH, Schumann G, Snieder H, Sternberg MJ, Stolk RP, Thomas HC, Thorsteinsdóttir U, Uda M, Waeber G, Wareham NJ, Waterworth DM, Watkins H, Whitfield JB, Witteman JC, Wolffenbuttel BH, Fox CS, Ala-Korpela M, Stefansson K, Vollenweider P, Völzke H, Schadt EE, Scott J, Järvelin MR, Elliott P, Kooner JS. Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. *Nat Genet* 2011; **43**: 1131-1138 [PMID: 22001757 DOI: 10.1038/ng.970]
- 37 **Smagris E**, BasuRay S, Li J, Huang Y, Lai KM, Gromada J, Cohen JC, Hobbs HH. Pnpla3^{I148M} knockin mice accumulate PNPLA3 on lipid droplets and develop hepatic steatosis. *Hepatology* 2015; **61**: 108-118 [PMID: 24917523 DOI: 10.1002/hep.27242]
- 38 **Huang Y**, Cohen JC, Hobbs HH. Expression and characterization of a PNPLA3 protein isoform (I148M) associated with nonalcoholic fatty liver disease. *J Biol Chem* 2011; **286**: 37085-37093 [PMID: 21878620 DOI: 10.1074/jbc.M111.290114]
- 39 **He S**, McPhaul C, Li JZ, Garuti R, Kinch L, Grishin NV, Cohen JC, Hobbs HH. A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J Biol Chem* 2010; **285**: 6706-6715 [PMID: 20034933 DOI: 10.1074/jbc.M109.064501]
- 40 **Fernández Gianotti T**, Burgueño A, Gonzales Mansilla N, Pirola CJ, Sookoian S. Fatty liver is associated with transcriptional downregulation of stearyl-CoA desaturase and impaired protein dimerization. *PLoS One* 2013; **8**: e76912 [PMID: 24098813 DOI: 10.1371/journal.pone.0076912]
- 41 **Silbernagel G**, Kovarova M, Cegan A, Machann J, Schick F, Lehmann R, Häring HU, Stefan N, Schleicher E, Fritsche A, Peter A. High hepatic SCD1 activity is associated with low liver fat content in healthy subjects under a lipogenic diet. *J Clin Endocrinol Metab* 2012; **97**: E2288-E2292 [PMID: 23015656 DOI: 10.1210/jc.2012-2152]
- 42 **Sookoian S**, Gianotti TF, Rosselli MS, Burgueño AL, Castaño GO, Pirola CJ. Liver transcriptional profile of atherosclerosis-related genes in human nonalcoholic fatty liver disease. *Atherosclerosis* 2011; **218**: 378-385 [PMID: 21664615 DOI: 10.1016/j.atherosclerosis.2011.05.014]
- 43 **Spassov DS**, Jurecic R. The PUF family of RNA-binding proteins: does evolutionarily conserved structure equal conserved function? *IUBMB Life* 2003; **55**: 359-366 [PMID: 14584586 DOI: 10.1080/15216540310001603093]
- 44 **Gieger C**, Geistlinger L, Altmaier E, Hrabé de Angelis M, Kronenberg F, Meitinger T, Mewes HW, Wichmann HE, Weinberger KM, Adamski J, Illig T, Suhre K. Genetics meets metabolomics: a genome-wide association study of metabolite profiles in human serum. *PLoS Genet* 2008; **4**: e1000282 [PMID: 19043545 DOI: 10.1371/journal.pgen.1000282]
- 45 **Kettunen J**, Tukiainen T, Sarin AP, Ortega-Alonso A, Tikkanen E, Lyytikäinen LP, Kangas AJ, Soininen P, Würtz P, Silander K, Dick DM, Rose RJ, Savolainen MJ, Viikari J, Kähönen M, Lehtimäki T, Pietiläinen KH, Inouye M, McCarthy MI, Jula A, Eriksson J, Raitakari OT, Salomaa V, Kaprio J, Järvelin MR, Peltonen L, Perola M, Freimer NB, Ala-Korpela M, Palotie A, Ripatti S. Genome-wide association study identifies multiple loci influencing human serum metabolite levels. *Nat Genet* 2012; **44**: 269-276 [PMID: 22286219 DOI: 10.1038/ng.1073]
- 46 **Shin SY**, Fauman EB, Petersen AK, Krumsiek J, Santos R, Huang J, Arnold M, Erte I, Forgetta V, Yang TP, Walter K, Menni C, Chen L, Vasquez L, Valdes AM, Hyde CL, Wang V, Ziemek D, Roberts P, Xi L, Grundberg E, Waldenberger M, Riehmek JB, Mohny RP, Milburn MV, John SL, Trimmer J, Theis FJ, Overington JP, Suhre K, Brosnan MJ, Gieger C, Kastenmüller G, Spector TD, Soranzo N. An atlas of genetic influences on human blood metabolites. *Nat Genet* 2014; **46**:

- 543-550 [PMID: 24816252 DOI: 10.1038/ng.2982]
- 47 **Chase BA**, Kankel DR. A genetic analysis of glutamatergic function in *Drosophila*. *J Neurobiol* 1987; **18**: 15-41 [PMID: 3106567 DOI: 10.1002/neu.480180104]
- 48 **Sardiello M**, Licciulli F, Catalano D, Attimonelli M, Caggese C. MitoDrome: a database of *Drosophila melanogaster* nuclear genes encoding proteins targeted to the mitochondrion. *Nucleic Acids Res* 2003; **31**: 322-324 [PMID: 12520013]
- 49 **Tomancač P**, Beaton A, Weiszmann R, Kwan E, Shu S, Lewis SE, Richards S, Ashburner M, Hartenstein V, Celniker SE, Rubin GM. Systematic determination of patterns of gene expression during *Drosophila* embryogenesis. *Genome Biol* 2002; **3**: RESEARCH0088 [PMID: 12537577]
- 50 **Cammarato A**, Ahrens CH, Alayari NN, Qeli E, Rucker J, Reedy MC, Zmasek CM, Gucek M, Cole RN, Van Eyk JE, Bodmer R, O'Rourke B, Bernstein SI, Foster DB. A mighty small heart: the cardiac proteome of adult *Drosophila melanogaster*. *PLoS One* 2011; **6**: e18497 [PMID: 21541028 DOI: 10.1371/journal.pone.0018497]
- 51 **Cermelli S**, Guo Y, Gross SP, Welte MA. The lipid-droplet proteome reveals that droplets are a protein-storage depot. *Curr Biol* 2006; **16**: 1783-1795 [PMID: 16979555 DOI: 10.1016/j.cub.2006.07.062]
- 52 **Alonso J**, Rodriguez JM, Baena-López LA, Santarén JF. Characterization of the *Drosophila melanogaster* mitochondrial proteome. *J Proteome Res* 2005; **4**: 1636-1645 [PMID: 16212416 DOI: 10.1021/pr050130c]
- 53 **Sookoian S**, Pirola CJ. The genetic epidemiology of nonalcoholic fatty liver disease: toward a personalized medicine. *Clin Liver Dis* 2012; **16**: 467-485 [PMID: 22824476 DOI: 10.1016/j.cld.2012.05.011]
- 54 **Sookoian S**, Pirola CJ. PNPLA3, the triacylglycerol synthesis/hydrolysis/storage dilemma, and nonalcoholic fatty liver disease. *World J Gastroenterol* 2012; **18**: 6018-6026 [PMID: 23155331 DOI: 10.3748/wjg.v18.i42.6018]
- 55 **Min HK**, Sookoian S, Pirola CJ, Cheng J, Mirshahi F, Sanyal AJ. Metabolic profiling reveals that PNPLA3 induces widespread effects on metabolism beyond triacylglycerol remodeling in Huh-7 hepatoma cells. *Am J Physiol Gastrointest Liver Physiol* 2014; **307**: G66-G76 [PMID: 24763554 DOI: 10.1152/ajpgi.00335.2013]
- 56 **Sookoian S**, Pirola CJ. NAFLD. Metabolic make-up of NASH: from fat and sugar to amino acids. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 205-207 [PMID: 24566880 DOI: 10.1038/nrgastro.2014.25]
- 57 **Mardinoglu A**, Agren R, Kampf C, Asplund A, Uhlen M, Nielsen J. Genome-scale metabolic modelling of hepatocytes reveals serine deficiency in patients with non-alcoholic fatty liver disease. *Nat Commun* 2014; **5**: 3083 [PMID: 24419221 DOI: 10.1038/ncomms4083]
- 58 **Wieme RJ**, Demeulenaere L. Enzyme assays in liver disease. *J Clin Pathol Suppl (Assoc Clin Pathol)* 1970; **4**: 51-59 [PMID: 4143914]
- 59 **Cassidy WM**, Reynolds TB. Serum lactic dehydrogenase in the differential diagnosis of acute hepatocellular injury. *J Clin Gastroenterol* 1994; **19**: 118-121 [PMID: 7963356]
- 60 **Rotenberg Z**, Weinberger I, Davidson E, Fuchs J, Harell D, Agmon J. Alterations in total lactate dehydrogenase and its isoenzyme-5 in hepatic disorders. *Ann Clin Lab Sci* 1990; **20**: 268-273 [PMID: 2403242]
- 61 **Zhou SL**, Gordon RE, Bradbury M, Stump D, Kiang CL, Berk PD. Ethanol up-regulates fatty acid uptake and plasma membrane expression and export of mitochondrial aspartate aminotransferase in HepG2 cells. *Hepatology* 1998; **27**: 1064-1074 [PMID: 9537447 DOI: 10.1002/hep.510270423]
- 62 **Clark JM**, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol* 2003; **98**: 960-967 [PMID: 12809815 DOI: 10.1111/j.1572-0241.2003.07486.x]
- 63 **Aigner E**, Hinz C, Steiner K, Rossmann B, Pflieger J, Hohla F, Steger B, Stadlmayr A, Patsch W, Datz C. Iron stores, liver transaminase levels and metabolic risk in healthy teenagers. *Eur J Clin Invest* 2010; **40**: 155-163 [PMID: 20050877 DOI: 10.1111/j.1365-2362.2009.02238.x]
- 64 **Sookoian S**, Castaño GO, Scian R, Mallardi P, Fernández Gianotti T, Burgueño AL, San Martino J, Pirola CJ. Genetic variation in transmembrane 6 superfamily member 2 and the risk of nonalcoholic fatty liver disease and histological disease severity. *Hepatology* 2014; Epub ahead of print [PMID: 25302781 DOI: 10.1002/hep.27556]
- 65 **Holmen OL**, Zhang H, Fan Y, Hovelson DH, Schmidt EM, Zhou W, Guo Y, Zhang J, Langhammer A, Løchen ML, Ganesh SK, Vatten L, Skorpen F, Dalen H, Zhang J, Pennathur S, Chen J, Platou C, Mathiesen EB, Wilsgaard T, Njølstad I, Boehnke M, Chen YE, Abecasis GR, Hveem K, Willer CJ. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. *Nat Genet* 2014; **46**: 345-351 [PMID: 24633158 DOI: 10.1038/ng.2926]
- 66 **Josekutty J**, Iqbal J, Iwawaki T, Kohno K, Hussain MM. Microsomal triglyceride transfer protein inhibition induces endoplasmic reticulum stress and increases gene transcription via Irf1/cjun to enhance plasma ALT/AST. *J Biol Chem* 2013; **288**: 14372-14383 [PMID: 23532846 DOI: 10.1074/jbc.M113.459602]
- 67 **Thulin P**, Raftar I, Stockling K, Tomkiewicz C, Norjavaara E, Aggerbeck M, Hellmold H, Ehrenborg E, Andersson U, Cotgreave I, Glinghammar B. PPARα regulates the hepatotoxic biomarker alanine aminotransferase (ALT1) gene expression in human hepatocytes. *Toxicol Appl Pharmacol* 2008; **231**: 1-9 [PMID: 18455211 DOI: 10.1016/j.taap.2008.03.007]
- 68 **Beránek M**, Drsata J, Palicka V. Inhibitory effect of glycation on catalytic activity of alanine aminotransferase. *Mol Cell Biochem* 2001; **218**: 35-39 [PMID: 11330835]
- 69 **Salgado MC**, Metón I, Anemaet IG, Baanante IV. Activating transcription factor 4 mediates up-regulation of alanine aminotransferase 2 gene expression under metabolic stress. *Biochim Biophys Acta* 2014; **1839**: 288-296 [PMID: 24418603 DOI: 10.1016/j.bbagr.2014.01.005]
- 70 **Coss CC**, Bauler M, Narayanan R, Miller DD, Dalton JT. Alanine aminotransferase regulation by androgens in non-hepatic tissues. *Pharm Res* 2012; **29**: 1046-1056 [PMID: 22167351 DOI: 10.1007/s11095-011-0649-5]
- 71 **Jadhao SB**, Yang RZ, Lin Q, Hu H, Anania FA, Shuldiner AR, Gong DW. Murine alanine aminotransferase: cDNA cloning, functional expression, and differential gene regulation in mouse fatty liver. *Hepatology* 2004; **39**: 1297-1302 [PMID: 15122758]
- 72 **Reagan WJ**, Yang RZ, Park S, Goldstein R, Brees D, Gong DW. Metabolic adaptive ALT isoenzyme response in livers of C57/BL6 mice treated with dexamethasone. *Toxicol Pathol* 2012; **40**: 1117-1127 [PMID: 22609950 DOI: 10.1177/0192623312447550]
- 73 **Liu R**, Pan X, Whittington PF. Increased hepatic expression is a major determinant of serum alanine aminotransferase elevation in mice with nonalcoholic steatohepatitis. *Liver Int* 2009; **29**: 337-343 [PMID: 18710424 DOI: 10.1111/j.1478-3231.2008.01862.x]
- 74 **Yuan X**, Waterworth D, Perry JR, Lim N, Song K, Chambers JC, Zhang W, Vollenweider P, Stirraddel H, Johnson T, Bergmann S, Beckmann ND, Li Y, Ferrucci L, Melzer D, Hernandez D, Singleton A, Scott J, Elliott P, Waeber G, Cardon L, Frayling TM, Kooner JS, Mooser V. Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. *Am J Hum Genet* 2008; **83**: 520-528 [PMID: 18940312 DOI: 10.1016/j.ajhg.2008.09.012]
- 75 **Park TJ**, Hwang JY, Go MJ, Lee HJ, Jang HB, Choi Y, Kang JH, Park KH, Choi MG, Song J, Kim BJ, Lee JY. Genome-wide association study of liver enzymes in Korean children. *Genomics Inform* 2013; **11**: 149-154 [PMID: 24124411 DOI: 10.5808/GI.2013.11.3.149]

- 76 **Shen H**, Damcott C, Shuldiner SR, Chai S, Yang R, Hu H, Gibson Q, Ryan KA, Mitchell BD, Gong DW. Genome-wide association study identifies genetic variants in GOT1 determining serum aspartate aminotransferase levels. *J Hum Genet* 2011; **56**: 801-805 [PMID: 21900944 DOI: 10.1038/jhg.2011.105]
- 77 **Vanburen V**, Chen H. Managing biological complexity across orthologs with a visual knowledgebase of documented biomolecular interactions. *Sci Rep* 2012; **2**: 1011 [PMID: 23264875 DOI: 10.1038/srep01011]
- 78 **Suhre K**, Shin SY, Petersen AK, Mohney RP, Meredith D, Wägele B, Altmaier E, Deloukas P, Erdmann J, Grundberg E, Hammond CJ, de Angelis MH, Kastenmüller G, Köttgen A, Kronenberg F, Mangino M, Meisinger C, Meitinger T, Mewes HW, Milburn MV, Prehn C, Raffler J, Ried JS, Römisch-Margl W, Samani NJ, Small KS, Wichmann HE, Zhai G, Illig T, Spector TD, Adamski J, Soranzo N, Gieger C. Human metabolic individuality in biomedical and pharmaceutical research. *Nature* 2011; **477**: 54-60 [PMID: 21886157 DOI: 10.1038/nature10354]

P- Reviewer: del Giudice EM, Duryee MJ, Mascitelli L, Tarquini R
S- Editor: Ma YJ **L- Editor:** A **E- Editor:** Liu XM





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



ISSN 1007-9327

