Liver enzymes, metabonomics 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 tran-saminase; Glutamic acid; Metabolism; Nonalcoholic fatty liver; Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Gene variants; Single nucleotide polymorphisms; PNPLA3; Genetics; Metabolomics; Metabolic
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\[6\], 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-1 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...
In addition, supporting the notion that elevation of ALT levels does not necessarily denote hepatocellular damage, Kechagias et al. showed that fast-food-based hyper-alimentation, when followed for four weeks, was associated with pathological serum ALT levels. Notably, the authors showed that the significant elevation of aminotransferases was not related to the development of liver steatosis. This clinical finding reinforces the hypothesis that an increase in the ALT enzymatic activity is an adaptive response to the liver metabolic demands. Table 2 summarizes the main features of ALT1 and ALT2, including novel aspects on their biological function and gene regulation, such as modulation by miR-122 to enhance ALT enzymatic activity, as recently reported by our group.

Glutamate-oxaloacetate 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 = mercaptoppyruvate + L-glutamate. GOT1 aliases are cysteine aminotransferase and transaminase A. In addition, the aspartate aminotransferase activity is involved in hepatic glucose synthesis during starvation. 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. 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 %.

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 %.

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 | CCR1 | 9q34.11 | 3 |
| Kynurenine--oxoglutarate transaminase 3 | CCR1L2 | 1p22.2 | 3 |
| Probable inactive 1-aminocyclopropane-1-carboxylate synthase-like protein 2 | ACCSL | 13p11.2 | 1 |
| Putative aspartate aminotransferase, cytoplasmic 2 | GPT1L1 | 8p11.23 | 1 |
| Tyrosine aminotransferase | TAT | 16q22.2 | 1 |
### 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 | Entrez gene: 84706 |
| 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 | There are 13 regulatory elements located in the region of GPT2 |
| Protein features | Size: 496 amino acids; 54637 Da | Size: 523 amino acids; 57904 Da |
| Cellular localization in human cells | Cofactor: Pyridoxal phosphate | Cofactor: Pyridoxal phosphate |
| Tissue expression in humans | Subunit: Homodimer | Subunit: Homodimer (By similarity) |
| Tissue expression in rodents | Cytosol of hepatocytes | ER and mitochondrial fraction |
| Measurement in plasma (catalytic activity) | Represents 90% of total ALT in circulation | Represents 10% of total ALT in circulation |
| Tissue expression in humans (Evidence: WB: Liver and kidney) | Evidence: WB and IHQ (protein): Pancreas (islets of Langerhans), brain, adrenal gland, skeletal muscle, heart (cardiomyocytes) | Evidence: WB and IHQ (protein): Pancreas (islets of Langerhans), brain, adrenal gland, skeletal muscle, heart (cardiomyocytes) |
| 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 | ALT1 catalytic activity is inhibited by the effect of glycation |
| Biological meaning in human disease | NAFLD: ALT1 represented 94% of total ALT levels in circulation | NAFLD: ALT2 represented 6% of total ALT levels in circulation |
| Biological meaning in experimental models of disease | HCV: High levels in circulation of ALT1 (about 5-fold increase as compared to the controls) | HCV: Moderate levels in circulation of ALT1 (about 2.5 fold increase as compared to the controls) |

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.
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(22). 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(22). 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), but also a wide spectrum of chronic liver diseases, as recently highlighted(30), including alcoholic liver disease(31), viral hepatitis C(32) and B(33) and hepatocellular carcinoma(34). Interestingly, the rs738409 located in PNPLA3 reached the most significant p value for association with liver enzyme levels \((1.2 \times 10^{-8})\) in the larger GWAS performed in Caucasians(35). 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 shown in Table 4.

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**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(23) | n = 61089 Adults | Affymetrix, Illumina and perlegen sciences arrays | About 2.6 million directly genotyped or imputed autosomal SNPs | Plasma levels of ALT | HSD17B13, MAPK10, TRIB1, CPNI, PNPLA3, SAMM50 |
| Yuan et al(24) | Initial study n ~ 7715 | Affymetrix | - | Plasma levels of ALT | CHLIK, PNPLA3, SAMM50, CPNI |
| Park et al(25) | n = 532 | Illumina HumanOmni1-Quad BeadChip | 747076 SNPs | Plasma levels of ALT | ST6GALNAC3, MMADHC, CDDC102B, RGSS, BRD7, GALNT13, SIRPA, CD93, SLC9A11, ADAMTS9, CELF2 |
| Shen et al(26) | n = 866 | Affymetrix GeneChip Human Mapping 500 K Array set | 500568 SNPs | Plasma levels of AST | CYB5APS, CELF2, GOT1, ST6GALNAC3, ADAMTS9, THSD7B, EIF4A1P1, RORB2, THSD7B, GOT1 |

GWAS: Genome-wide association studies; SNPs: Single nucleotide polymorphisms; ALT: Alanine-aminotransferase; AST: Aspartate-aminotransferase.
### 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^{-8} | HSD17B13/MAPK10 | Oxidoreductase involved in the metabolism of steroid hormones, prosta glandinos, retinoids, lipids and xenobiotics |
|  | rs2954021 | Intergenic | 5.3 × 10^{-8} | 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^{-7} | CPN1 | A plasma metallo-protease that cleaves basic amino acids from the C terminal of peptides and proteins |
|  | rs11597390 | Intergenic | 2.9 × 10^{-7} | PNPLA3 | A cytosolic sphingolipid transfer protein that mediates triacylglycerol lipase and triacylglycerol lipase synthesis |
|  | rs7281135 | Intergenic | 1.2 × 10^{-6} | SAMM50 | Component of the sorting and assembly machinery (SAM) of the mitochondrial outer membrane |
|  | rs2143571 | Intergenic | 8.2 × 10^{-12} | 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 |
|  | rs11597086 | Intergenic | 4.5 × 10^{-7} | ST6GALNAC3 | Transfer sialic acids from CMP-sialic acid to terminal positions of carbohydrate groups in glycoproteins and glycolipids |
|  | rs23700127 | Intergenic | 3.7 × 10^{-7} | MMADHC | Mitochondrial protein that is involved in an early step of vitamin B12 metabolism |
|  | rs1939893 | Intergenic | 3.40 × 10^{-10} | CCDC102B | Unknown |
|  | rs51253879 | Intergenic | 3.97 × 10^{-10} | RG55 | Member of the regulators of G protein signaling (RGS) family |
|  | rs9941219 | Intergenic | 4.06 × 10^{-10} | BRD7 | Member of the bromodomain-containing protein family |
|  | rs751160 | Intergenic | 4.36 × 10^{-10} | GALNT13 | Member of the glycosyltransferase 2 family; catalyzes the initial reaction in glycoprotein and glycolipid synthesis; catalyzes the initial reactions in the synthesis of glycoproteins and glycolipids; catalyzes the initial reaction in the synthesis of glycoproteins and glycolipids; catalyzes the initial reaction in the synthesis of glycoproteins and glycolipids; catalyzes the initial reaction in the synthesis of glycoproteins and glycolipids |
|  | rs6035126 | Intergenic | 4.94 × 10^{-10} | SIRPA | Receptor-type transmembrane glycoproteins involved in the negative regulation of receptor tyrosine kinase-coupled signaling processes |
|  | rs13433286 | Intergenic | 5.64 × 10^{-10} | CD93 | Cell-surface glycoprotein and type 1 membrane protein |
|  | rs844917 | Intergenic | 5.98 × 10^{-10} | SLC9A11 | Mediates zinc uptake |
|  | rs8101637 | Intergenic | 6.01 × 10^{-10} | ADAMTS9 | Disintegrin and metalloprotease with thrombospondin motifs |
|  | rs596406 | Intergenic | 7.18 × 10^{-10} | CEF2 | RNA-binding protein implicated in the regulation of several post-transcriptional events |
|  | rs11597390 | Intergenic | 2.80 × 10^{-14} | ST6GALNAC3 | Transfer sialic acids from CMP-sialic acid to terminal positions of carbohydrate groups in glycoproteins and glycolipids |
|  | rs23700127 | Intergenic | 1.49 × 10^{-10} | CD93 | Mitochondrial protein that is involved in an early step of vitamin B12 metabolism |
|  | rs652946 | Intergenic | 5.7 × 10^{-10} | PNPLA3 | Pseudogene |
|  | rs596406 | Intergenic | 3.69 × 10^{-10} | CELF2 | Cells | Explained previously |
|  | rs76850951 | Intergenic | 8.55 × 10^{-10} | GOT | Biosynthesis of L-glutamate from L-aspartate or L-cysteine |
|  | rs7179293 | Intergenic | 2.80 × 10^{-14} | CHUK | Explained previously |
|  | rs4949718 | Intergenic | 1.49 × 10^{-10} | ST6GALNAC3 | Explained previously |
|  | rs80311637 | Intergenic | 1.85 × 10^{-10} | PNPLA3 | Explained previously |
|  | rs596406 | Intergenic | 2.80 × 10^{-14} | PNPLA3 | Pseudogene |
|  | rs7618097 | Intergenic | 3.75 × 10^{-10} | PNPLA3 | Pseudogene |
|  | rs11597390 | Intergenic | 6.16 × 10^{-10} | ROBO1 | Neuronal development |

*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 beta-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; RG55: Regulator of G-protein signaling 5; BRD7: Bromodomain containing 7; GALNT13: Polypeptide N-acetylgalactosaminyltransferase 13; SIRPA: Signal-regulatory protein alpha; CD93: CD93 molecule; SLC9A11: Solute carrier family 39, member 11; ADAMTS9: ADAM metalloproteinase with thrombospondin type 1 motif, 9; CYB5AP5: Cytochrome b5 type A pseudogene; CELF2: CELF2, Elav-like family member 2; GOT1: Glutamic-oxaloacetic transaminase 1, soluble; THSD7B: thrombospondin, type 1, domain containing 7; 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).
SYSTEMS BIOLOGY APPROACHES TO EXPLORING A PUTATIVE CONNECTION BETWEEN THE SIGNIFICANTLY ASSOCIATED LOCUS WITH LIVER TRANSAMINASINES

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.htm. 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.

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, and 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 PP-4RC1β (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
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\cite{44}. Examples of GWAS relevance are the discovery of SNPs in GLS2 (glutamine synthase 2) associated with glutamine levels, such as the rs2638315\cite{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\cite{46}. Shin et al\cite{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 phenylalanine levels\cite{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: RNU6-115SP ENSG00000200424). Genetic regulation of these metabolites may indicate that GOT2 would be not only involved in the maintenance of the equilibrium of amino acids.
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\(\backslash\)Got2, is involved in glutamate\(^{[47]}\) and aspartate metabolic process\(^{[48]}\). Moreover, tissue localization yielded evidence, indicating that Dmel\(\backslash\)Got2 is expressed in embryonic larval fat body and midgut\(^{[49]}\), and in adult heart\(^{[50]}\). Furthermore, subcellular localization showed evidence of Dmel\(\backslash\)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 epandrosterone 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 \(\times\) 10\(^{-10}\)).

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/gamaglutamyltyrosine, docosapentaenoate (n3 DPA; 22:5n3)/docosapentaenoate (n3 DPA; 22:5n3)/docosapentaenoate (n3 DPA; 22:5n3) (Table 5). Remarkably, docosapentaenoic 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\(^{[55]}\). 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\(^{[59]}\). 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)\(^{[58]}\).

Wiene 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\(^{[59]}\). 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\(^{[38]}\), 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\(^{[38]}\) 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.

![Biological half-life value of liver enzymes in the plasma (h)](image-url)

**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\(^{[38]}\). 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.

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**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 |
|------------|-----------------|-----------------|-------------------------|
| rs1243959  | 0.657           | Cholesterol/gamma-glutamyltyrosine | 7.76 × 10\(^{-4}\) |
| rs2076211  | 0.657           | Cholesterol/gamma-glutamyltyrosine | 1.19 × 10\(^{-3}\) |
| rs2076211  | 0.657           | Aspartylphenylalanine/docosapentaenoate (n3 DPA; 22:5n3) | 5.26 × 10\(^{-4}\) |
| rs2294922  | 0.657           | Docosapentaenoate (n3 DPA; 22:5n3)/eicosapentaenoate (EPA; 20:5n3) | 4.64 × 10\(^{-7}\) |
| rs2076211  | 0.657           | Docosapentaenoate (n3 DPA; 22:5n3)/phenylacetylglutamine | 1.43 × 10\(^{-4}\) |
| rs2281135  | 0.609           | Glycocolate/levulinate (4-oxovalerate) | 3.26 × 10\(^{-4}\) |
| rs1010225  | 0.609           | Docosapentaenoate (n3 DPA; 22:5n3)/phenylacetylglutamine | 1.98 × 10\(^{-3}\) |
| rs296303   | 0.609           | Aspartylphenylalanine/docosapentaenoate (n3 DPA; 22:5n3) | 1.31 × 10\(^{-3}\) |

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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 R² (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.
and the risk of metabolic disturbances in adolescents that deserves further follow-up.

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

In conclusion, genomic, transcriptomic, proteomic, and metabolomic information has changed the classical conception of the meaning that serum concentrations of ALT and AST 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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