Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma

This is a pre print version of the following article:

Original Citation:

Availability:
This version is available http://hdl.handle.net/2318/1562250 since 2017-10-24T15:35:08Z

Published version:
DOI:10.1038/ng.970

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Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma

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Abstract
Concentrations of liver enzymes in plasma are widely used as indicators of liver disease. We carried out a genome-wide association study in 61,089 individuals, identifying 42 loci associated with concentrations of liver enzymes in plasma, of which 32 are new associations (P = 10−8 to P = 10−190). We used functional genomic approaches including metabonomic profiling and gene expression analyses to identify probable candidate genes at these regions. We identified 69 candidate genes, including genes involved in biliary transport (ATP8B1 and ABCB11), glucose, carbohydrate and lipid metabolism (FADS1, FADS2, GCKR, JMJD1C, HNF1A, MLXIPL, PNPLA3, PPP1R3B, SLC2A2 and TRIB1), glycoprotein biosynthesis and cell surface glycochemistry (ABO, ASGR1, FUT2, GPD1 and ST3GAL4), inflammation and immunity (CD276, CDH6, GCKR, HNF1A, HPR, ITGA1, RORA and STAT4) and glutathione metabolism (GSTT1, GSTT2 and GGT), as well as several genes of uncertain or unknown function (including ABHD12, EFHD1, EFNA1, EPHA2, MICAL3 and ZNF827). Our results provide new insight into genetic mechanisms and pathways influencing markers of liver function.

High concentrations of liver enzymes in plasma are observed in liver injury caused by multiple insults including alcohol misuse, viral and other infections, metabolic disorders, obesity, autoimmune disease and drug toxicity. High liver enzyme concentrations are associated with increased risk of cirrhosis2, hepatocellular carcinoma3, type 2 diabetes4 and cardiovascular disease5. Abnormal liver function is a common reason for terminating new clinical therapeutic agents, representing a major challenge for the global pharmaceutical industry6. Liver enzyme concentrations in plasma are highly heritable7, suggesting an important role for genetic factors.

We carried out a genome-wide association study (GWAS) in 61,089 research participants to identify genetic loci influencing liver function measured by concentrations of alanine transaminase (ALT), alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) in blood. ALT is mainly a marker of hepatocellular damage1, and may also be high in obesity and fatty liver disease8. ALP is a marker of biliary obstruction, and is also released from bone, intestine, leucocytes and other cells1. GGT is sensitive to most kinds of liver insult, particularly alcohol1. Our study design is summarized in Figure 1. Characteristics of participants, genotyping arrays and quality control measures are summarized in Supplementary Tables 1–4. Genome-wide significance was inferred at P < 1 × 10−8, allowing a Bonferroni correction for ~106 independent SNPs tested9, and for three separate liver markers; the latter is a conservative adjustment given the correlations between concentrations of the three liver markers (r = 0.19–0.64) and their association test results (r = 0.02–0.19; Supplementary Table 5).

We found 1,304 SNPs associated with one or more liver markers at P < 1 × 10−7 across 42 genetic loci (Table 1 and Fig. 2). At 35 of these loci, one or more SNPs reached genome-wide significance (P < 1 × 10−8;
Supplementary Table 6); at the other seven genetic loci, the top-ranking SNP reached genome-wide significance after further testing in an additional sample of 12,139 research participants (Supplementary Table 7). Regional plots for each of the genetic loci are shown in Supplementary Figures 1–3. Common variants at chromosome 8q24 were associated with both ALP and ALT, and variants at chromosome 19q13 were associated with both ALP and GGT, at P < 1 × 10−8. Sixteen loci associated with one liver marker at P < 10−8 showed additional associations with a second marker at P < 6 × 10−4 (corresponding to P < 0.05 after Bonferroni correction for testing 42 loci against two alternate liver markers; Supplementary Fig. 4 and Supplementary Table 8). The loci previously reported to be associated with liver markers in GWASs were replicated in the current study, except for variants at the ALDH2 locus reported in Japanese populations, which have low allele frequency in European populations10,11.

We used coding variation, expression quantitative trait loci (eQTL) and GRAIL analyses to identify possible candidate genes at the 42 loci associated with liver enzymes (Table 1 and Supplementary Table 9). There are 19 nonsynonymous SNPs (nsSNPs) that are in linkage disequilibrium (LD) with one or more of the sentinel SNPs at r2 ≥ 0.5 in the HapMap phase II CEU data set12 (see URLs), representing a ~3.5-fold enrichment compared with the number expected under the null hypothesis (P = 0.004). We considered the gene containing the nsSNP to be a strong candidate when (i) the nsSNP and the sentinel SNPs were in LD (r2 > 0.5) and (ii) there was no evidence for heterogeneity of effect on phenotype. The genes with coding variants identified as candidates for mediating the observed associations with liver markers (Supplementary Table 10) encode proteins involved in biliary transport (ATP8B1)13, cell surface glycochemistry, endoplasmic trafficking and susceptibility to gastrointestinal infection (FUT2 and GLPD1)14,15, carbohydrate and lipid metabolism, including susceptibility to type 2 diabetes (GCKR, HNF1A and SLC2A2)16–18 and inflammation as measured by circulating concentrations of C-reactive protein (CRP) (GCKR and HNF1A)19. Mutations in ATP8B1 are responsible for progressive familial intrahepatic cholestasis and are associated with high GGT concentrations20; the coding variant identified is predicted to be nonconservative (Supplementary Fig. 5). At chromosome 14q32, rs944002 is in LD (r2 = 0.86) with two nsSNPs in C14orf73, a gene strongly expressed in liver. C14orf73 has strong sequence homology with SEC6, a protein that interacts with the actin cytoskeleton and vesicle transport machinery21. Of the two nsSNPs reported in C14orf73, p.Arg77Trp is predicted to be a nonconservative change from a polar basic residue to a nonpolar hydrophobic residue (Supplementary Fig. 5).

We repeated the search for coding variants using available results from the 1000 Genomes Project22 (see URLs) and identified coding variants in two additional genes, NBPF3 (chromosome 1p36.12) and MLXIPL (chromosome 7q11). Both genes are separately implicated as candidates for genes mediating the associations of sentinel SNPs with liver markers through eQTL analyses.

We examined the association of the sentinel SNPs with eQTL data from liver, fat and peripheral blood leukocytes23–25 (Supplementary Tables 11–14). We tested SNPs for association with expression of nearby (within 1 Mb) genes (at P < 0.05 after Bonferroni correction for number of SNP expression associations tested). When we identified probable eQTLs, we tested whether the sentinel SNP and the SNP most closely associated with the eQTL were coincident (r2 > 0.5 and absence of heterogeneity at the phenotype or eQTL). This strategy identified eQTLs at 23 of the 42 loci, representing genes implicated in glutathione metabolism and drug detoxification (GSTT1 and GGT1), carbohydrate and lipid metabolism (MLXIPL, PPP1R3B, FADS1 and FADS2), cell signaling (ABHD12 and EPHA2) and inflammation and immunity (STAT4, MAPK10, CD276 and HPR). The functions of the other candidate genes identified by eQTLs (including EFHD1, MICAL3, DENND2D, CEPT1, MLIP (also known as C6orf142) and RSG1 (also known as C1orf89)) are poorly understood.
We also carried out a literature analysis using the GRAIL algorithm26 (see URLs), initially using the 2006 data set to avoid studies of the GWAS era. At chromosome 2q24, GRAIL identified ABCB11 as the most plausible candidate (Supplementary Table 15). ABCB11 activity is a major determinant of bile formation and bile flow27; mutations in ABCB11 cause progressive familial intra-hepatic cholestasis type 2 and are associated with increased risk of hepatocellular carcinoma28,29. We repeated the GRAIL analysis using the 2010 PubMed data set. This also identified ABCB11 as the plausible candidate at chromosome 2q24 but additionally identified ABO, GCKR, MLXIPL and PNPLA3 as probable candidates at other loci (Supplementary Table 15), replicating our findings from coding variant and eQTL analyses.

Through our coding variant, expression and GRAIL analyses, we identified 44 genes as strong candidates at the 42 loci associated with concentrations of liver enzymes in plasma. We also considered the gene nearest to the sentinel SNP at each locus to be a potential candidate. Together these approaches identified 69 candidate genes. Pathway analyses showed subnetworks of closely interconnected genes (Supplementary Fig. 6) from core metabolic pathways and processes including carbohydrate metabolism, insulin signaling and diabetes (GCKR, SLC2A2, PPP1R3B, FUT2, ALDOB, HNF1A and MLXIPL), lipid metabolism (CEPT1, FADS1, FADS2, HNF1A, PNPLA3 and ALDH5A1), glycosphingolipid biosynthesis and glycosylation (ST3GAL4, FUT2 and ABO) and glutathione metabolism (ALDH5A5, GGT1 and GSTT1).

Of the 42 liver marker loci, 24 have been reported to be associated with other phenotypes in genome-wide studies (Supplementary Table 16). At 12 of the loci, the lead SNP for the liver marker and the phenotype are the same or in LD at r2 ≥ 0.5, suggesting shared biological pathways. The phenotypes include Crohn’s disease, pancreatic carcinoma, type 2 diabetes, waist circumference and concentrations of glucose, insulin, total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, fatty acids, uric acid and C-reactive protein. At other loci, the sentinel SNP from the liver marker GWAS and the lead SNP in the US National Human Genome Research Institute (NHGRI) catalog30 (see URLs) are in low LD, suggesting that these likely represent different underlying mechanisms. We also ascertained the relationships of the 42 loci with quantitative anthropometric and metabolic traits in published genome-wide meta-analyses (Supplementary Table 17). We found that the loci associated with liver enzymes are enriched in SNPs associated with lipid concentrations, fasting glucose and inflammation as measured by CRP.

We used metabonomic profiling, the systematic characterization of a metabolite panel, to better understand the relationships of the 42 liver enzyme loci with intermediary and lipoprotein metabolism. We carried out quantitative nuclear magnetic resonance (NMR) spectroscopy on serum samples from 6,516 participants from the London Life Sciences Population31 (LOLIPOP) and Northern Finland Birth Cohort 1966 (ref. 32; NFBC1966) studies. Significance was inferred at P < 1 × 10−5, corresponding to P < 0.05 after Bonferroni correction for the 42 independent SNPs tested, and for the 69 primary NMR measures. At chromosomes 2p23 (C2orf16 and GCKR) and 8q24 (TRIB1), effect alleles of the sentinel SNPs are associated with high very low-density lipoprotein, intermediate-density lipoprotein and LDL concentration and VLDL particle size, high lipoprotein triglyceride and cholesterol concentration, omega-3 and omega-6 fatty acid concentrations, and concentrations of metabolic substrates citrate, pyruvate and branch chain amino acids (Fig. 3). At chromosome 12q24 (HNF1A), rs7310409 is associated with lipoprotein concentration and composition, and with tyrosine concentrations. At chromosomes 11q12 (C11orf10, FADS1 and FADS2) and 8p23 (PPP1R3B), the effect alleles are associated with low concentrations of cholesterol and HDL cholesterol and with low concentrations of omega-3 and other unsaturated fatty acids. Our results from the
NMR confirm and extend previous studies using mass spectroscopy, which showed strong association of GCKR and FADS1 with absolute and relative abundances of polyunsaturated fatty acids33,34.

We examined the contribution of the 42 genetic loci to concentrations of liver enzymes in plasma among the 8,112 participants of the LIFELINES population study35. SNPs at 41 loci showed consistent direction of effect (P = 4 × 10−13, sign test; Supplementary Table 18). Together the SNPs associated with each liver enzyme account for 0.1%, 3.5% and 1.9% of population variation in plasma concentrations of ALT, ALP and GGT, respectively (Supplementary Table 19). We then constructed a SNP score as the unweighted sum of the effect allele counts for the SNPs associated with each liver marker. Participants in the top quartile of distribution for SNP score for ALT, ALP or GGT were ~1.4, ~2.4 and ~1.8 times more probable to have greater than the upper limit of normal concentrations of ALT, ALP and GGT, and on average had concentrations of ALT, ALP and GGT that were 7%, 13% or 26% higher, respectively, than participants in the lowest quartile of SNP score (Supplementary Table 19).

Finally we tested the relationship of the liver enzyme–associated loci with the presence of structural changes in the liver indicative of hepatic steatosis, as determined by computerized axial tomography (CT) scanning in a population sample of 9,610 participants of the Genetics of Liver Disease (GOLD) study36. SNPs at five loci were associated with hepatic steatosis at P < 0.05, including PNPLA3, PPP1R3B, GCKR, TRIB1, HNF1A and SOX9 loci (Supplementary Table 20); of these, PNPLA3, PPP1R3B and GCKR were associated with hepatic steatosis at P < 0.0012 (that is, P < 0.05 after Bonferroni correction for 42 loci).

We identify 42 independent loci associated with ALP, ALT or GGT and 69 genes as candidates for the associations observed (Supplementary Table 9). The candidate genes include ATP8B1 and ABCB11, encoding biliary transporters with a key role in bile formation and flow20,37, and many genes involved in carbohydrate and lipid metabolism, including GCKR, MLXIPL, SLC2A2, HNF1A, PNPLA3, FADS1, FADS2 and PPP1R3B17,38,39. PNPLA3, PPP1R3B and GCKR influence accumulation of hepatic triglycerides40,41. We identify GSTT1, GSTT2 and GGT as candidates encoding key enzymes in glutathione synthesis and drug metabolism42,43; these observations may be relevant to pharmacogenetics and drug development. We also identify a set of genes involved in inflammation and immunity, including CD276, CDH6, GCKR, HPR, ITGA1, MAPK10, RORA and STAT4. Whether these genes influence hepatic inflammatory responses to accumulation of triglycerides, viral infection or other exogenous challenges remains to be determined. Finally we identify a set of genes involved in glycoprotein biology, including ABO, ASGR1, FUT2, GPLD1 and ST3GAL4. The products of these genes influence synthesis, cell surface binding and turnover of glycoproteins. These pathways are linked to susceptibility to pancreatic44 and gastric malignancy45, intestinal and other infections46 and vitamin B12 metabolism47. The pleiotropic nature of the genes we identified suggests that their relationships with ALP, ALT or GGT may also be mediated by pathways operating outside of the liver.

In summary, we report a GWAS for concentrations of liver enzymes in plasma, providing new insight into the genetic variation and pathways influencing ALP, ALT and GGT. Our findings provide the basis for further studies investigating the biological mechanisms involved in liver injury.

METHODS
Participants
Genome-wide association was done among 61,089 participants from the following published studies: the Australian Twin cohort (n = 425)\(^4\); the British Genetics of Hypertension study (BRIGHT, n = 1,955)\(^4\); the Lausanne Cohort (CoLaus, n = 5,636)\(^5\); deCODE genetics (n = 12,572)\(^6\); the Fenland study (n = 1,397)\(^7\); the Finnish Twin cohort study (FinnTwin, n = 32)\(^8\); the Framingham Heart Study (n = 2,869)\(^9\); the Monica/KORA Augsburg study (KORA, n = 1,809)\(^10\); the London Life Sciences Population study (LOLIPOP, n = 10,338)\(^11\); the Northern Finland Birth Cohort 1966 (NFBC1966, n = 4,562)\(^12\); the Netherlands Study of Depression and Anxiety (NEDS, n = 1,724)\(^13\); the Netherlands Twin study (n = 1,721)\(^14\); the Precocious Coronary Artery Disease study (Procardis, n = 1,239)\(^15\); the Rotterdam Study 1 (RS1, n = 4,312)\(^16\); the SardiNIA study (n = 4,302)\(^17\); the Study of Health in Pomerania (SHIP, n = 4,101)\(^18\) and the TwinsUK study (n = 2,256)\(^19\). Sample sizes for ALT, ALP and GGT genome-wide analyses were 45,596, 56,415 and 61,089, respectively. Further characteristics of the genome-wide association cohorts are listed in Supplementary Note and Supplementary Tables 1 and 2. SNPs showing equivocal association with liver markers were further tested among 12,139 participants from the LOLIPOP study, with none included in the genome-wide study (Supplementary Table 4).

**Genotyping and quality control**

Genome-wide association scans were done using Affymetrix, Illumina and Perlegen Sciences arrays (Supplementary Table 3). Imputation of missing genotypes was done using phased haplotypes from HapMap build36 and dbSNP build 126. Imputed SNPs with minor allele frequency < 0.01 or low-quality score (r2 < 0.30 in MACH, or information score <0.3 in IMPUTE) were removed. This generated ~2.6 million directly genotyped or imputed autosomal SNPs. Genotyping for further testing was done by KASPar (K-Biosciences, LTD).

**Statistical analysis**

Plasma concentrations of ALT, ALP and GGT were log10 transformed to achieve approximate normality. SNPs were tested for association with liver markers by linear regression using an additive genetic model adjusted for age and sex. An additional term was included to indicate case status in case-control studies, and principal component scores (EIGENSTRAT) were used to adjust for substructure in studies of unrelated individuals (Supplementary Table 3). Test statistics were corrected for respective genomic control inflation factor (Supplementary Table 4) to adjust for residual population structure. Association analyses were carried out separately in each cohort followed by meta-analysis using weighted z scores. Meta-analysis P values were then corrected for the meta-analysis genomic control inflation factors. The GWAS had 80% power to detect SNPs associated with 0.1% of population variation in ALP and 0.06% of population variation in ALT and GGT at P < 5 × 10\(^{-7}\).

In the replication samples, SNP associations were tested by linear regression using an additive genetic model and adjustment for age and sex. Results were combined with findings from the genome-wide association cohorts, using the weighted z scores. Genome-wide significance was inferred at P < 1 × 10\(^{-8}\).

SNP effect sizes were estimated by inverse-variance meta-analysis in the genome-wide association cohorts and available replication cohorts using a fixed effects model.

**Coding variant analyses**
We identified coding SNPs within 1 Mb and in LD at \( r^2 > 0.5 \) with the sentinel liver SNPs using HapMap CEU II genotype data (see URLs). We tested for enrichment by permutation testing using 42 randomly selected SNPs from the ~2.6 million genotyped or imputed SNPs studied that had similar minor allele frequency ±0.02), number of nearby genes (±10%) and gene proximity (±20 kb) to the sentinel SNPs. We counted coding SNPs within 1 Mb and in LD at \( r^2 > 0.5 \) of the random SNPs; this was repeated 1,000 times to generate a distribution for expected, against which we compared the number observed (\( n = 19, P = 0.004 \)).

We considered a coding SNP to be a strong candidate for the observed association when it was in LD at \( r^2 > 0.5 \) with the sentinel SNP, with no evidence for heterogeneity of effect on phenotype (\( P > 0.05 \)). Using this approach, we identified 17 coding SNPs in 14 genes as candidates for mediating the observed associations with liver markers (Supplementary Table 10). We used PHYRE64 to model the molecular structure of the protein products and possible pathogenicity of the coding SNPs identified.

Expression analyses

The sentinel SNPs from the liver marker GWAS were tested for association with gene expression in 603 adipose and 745 peripheral blood samples from Icelandic subjects25, peripheral blood lymphocytes from 206 families of European descent (830 parents and offspring)23 and 960 human liver samples24. Sentinel SNPs were tested for association with transcript levels of genes within 1 Mb; significance was inferred at \( P < 0.05 \) after Bonferroni correction for number of SNP-transcript combinations tested. We then used the whole-genome genotype data to identify which SNP from the liver locus was most closely associated with the transcript of interest; we defined this as the transcript SNP. We tested whether the sentinel SNP and transcript SNP were coincident, defined as in LD at \( r^2 > 0.5 \), with no evidence for heterogeneity of effect between the SNPs on transcript expression or liver marker phenotype.

GRAIL

We carried out a PubMed literature analysis using GRAIL (see URLs)65 including all 42 sentinel SNPs simultaneously. We used the 2006 PubMed data set as the primary analysis (Supplementary Table 15) but repeated the analysis using the 2010 PubMed data set.

Network analyses

Network analyses were carried out using the Ingenuity Pathway Analysis tool66. P values for canonical pathways and functions were calculated from the observed number of candidate genes in the gene set, compared with the number expected under the null hypothesis and corrected (Bonferroni) for the number of pathways tested.

Overlap with other GWAS

We used the NHGRI30 catalog (see URLs) to identify other phenotypic associations (\( P < 5 \times 10^{-8} \)) located within 1 Mb of a the SNPs we identified as associated with liver enzymes (Supplementary Table 16). Previous studies reporting genetic variants influencing concentrations of liver enzymes in plasma were excluded. Pairwise LD with the sentinel liver marker SNP was determined using HapMap 2 CEU genotype data.

Phenotypic pleiotropy
Relationships of the selected 42 sentinel SNPs with anthropometric and metabolic traits relevant to liver function were tested in the following genome-wide meta-analyses (Supplementary Table 17): AlcGen Consortium, alcohol consumption67; ICBP-GWAS, systolic and diastolic blood pressure68; the Genetics of C-reactive Protein Study (CRP-Gen), C-reactive protein19; MAGIC, fasting glucose and related glycemic traits16; DIAGRAM+ Study, type 2 diabetes17; GIANT Consortium, body mass index69 and the Global Lipids Genetics Consortium, total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride concentrations70. Associations were tested in silico using results from the genome-wide association phase and adopting the phenotypic definitions applied in each study. We inferred association of SNP with phenotype at P < 0.0012, corresponding to P < 0.05 after Bonferroni correction for 42 loci. We tested whether phenotypes were enriched for association with liver marker SNPs using a binomial probability test.

Metabonomic analyses

We carried out quantitative NMR spectroscopy on serum samples from 2,269 LOLIPOP and 4,247 NFBC1966 participants with genome-wide data to investigate the relationships of the identified loci with lipoprotein and intermediary metabolism. NMR assays were carried out using a Bruker AVANCE III spectrometer operating at 500.36 MHz (1H observation frequency; 11.74 T) and equipped with an inverse selective SEI probe-head including an automatic tuning and matching unit and a z-axis gradient coil for automated shimming71,72. A BTO-2000 thermocouple was used for temperature stabilization of the sample at ~0.01 °C. The high-performance electronics enabled metabolite quantification without per-sample chemical referencing or double-tube systems. The NMR methodology provides information on lipoprotein subclass distribution and lipoprotein particle concentrations, low-molecular-mass metabolites such as amino acids, 3-hydroxybutyrate and creatinine, and detailed molecular information on serum lipids including free and esterified cholesterol, sphingomyelin, saturation, unsaturation, polyunsaturation and omega-3 fatty acids73. Associations of SNPs with metabolic measures were tested in each cohort separately using an additive genetic model and were adjusted for age, gender and principal components. Results for LOLIPOP and NFBC1966 were combined by inverse variance meta-analysis, and significance was inferred at P < 1 × 10−5 (corresponding to P < 0.05 after Bonferroni correction for the 42 independent SNPs tested and for 69 primary NMR measures).

Contribution of genetic loci identified to population variation in liver enzymes

This was investigated in the LifeLines Cohort Study35, a prospective population-based cohort study of 165,000 persons aged 18–90 living in The Netherlands, and independent of the genome-wide association discovery cohorts. Genotyping was carried out in representative samples of 8,112 participants (aged 47.8 ± 11.2, body mass index 26.2 ± 4.3 kg/m2 (mean ± s.d.), 43% male) using the Illumina CytoSNP12 array, and imputation of missing HapMap2 genotypes was done using Beagle 3.1.0. Liver markers were measured on a Roche/Hitachi Modular System (Roche Diagnostics). Mean ± s.d. concentrations of liver markers were 23.8 ± 16.8, 62.8 ± 18.4 and 26.3 ± 24.5 IU/l for ALT, ALP and GGT, respectively. The contribution of SNPs to population variation in liver markers was examined individually and in aggregate (Supplementary Tables 18 and 19). For the latter, SNP scores were calculated for each individual on the basis of the sum of effect (trait-raising) alleles present at each of the genetic loci identified.

Liver imaging for hepatic steatosis
Hepatic steatosis was assessed by CT scanning in 9,610 participants from four population cohorts primarily designed for investigation of cardiovascular disease and its risk factors, (i) AGES-Reykjavik (n = 4,772), (ii) the Amish study (n = 541), (iii) the Family Heart Study (n = 886) and (iv) the Framingham Study (n = 3,411)36. CT measurements, blind to participant characteristics, were calibrated against phantoms and inverse normally transformed. Genome-wide SNP data were available in each cohort with imputation of missing genotypes. SNP association with hepatic steatosis was tested in each cohort separately by linear regression with age, with age2 and gender as covariates and taking relatedness into account. Results were combined by fixed-effect inverse-variance meta-analysis (Supplementary Table 20).

Acknowledgments

We thank the many colleagues who contributed to collection and phenotypic characterization of the clinical samples, as well as genotyping and analysis of data. We also thank the research participants who took part in these studies. Major support for the work came from European Commission (FP5, FP6 and FP7); European Science Foundation; European Science Council; US NIH; US National Institute of Mental Health; US NIDDK; Genetic Association Information Network; US National Institute on Aging; US National Human Genome Research Institute; US NHLBI; UK NIHR; NIHR Comprehensive Biomedical Research Centre Imperial College Healthcare NHS Trust; NIHR Comprehensive Biomedical Research Centre Guy’s and St. Thomas’ NHS Trust; UK Biotechnology and Biological Sciences Research Council; UK MRC; British Heart Foundation; Wellcome Trust; Swiss National Science Foundation; Academy of Finland; Finnish Cardiovascular Research Foundation; Swedish Research Council; Swedish Heart-Lung Foundation; Helmholtz Zentrum München; German Research Center for Environmental Health; German Federal Ministry of Education and Research; German National Genome Research Network; Netherlands Organization for Scientific Research; Dutch Ministries of Economic Affairs, of Education, Culture and Science, for Health, Welfare and Sports; Netherlands Organization for Health Research and Development; Economic Structure Enhancing Fund of the Dutch government; Dutch Kidney Foundation; Dutch Diabetes Research Foundation; Dutch Brain Foundation; Dutch Research Institute for Diseases in the Elderly; Netherlands Genomics Initiative; Canadian Institutes for Health Research; Ontario Research Fund; The Barts and the London Charity; University Medical Center Groningen; University of Groningen; University of Oulu, Biocenter Oulu; University Hospital Oulu; Biocentrum Helsinki; Erasmus Medical Center and Erasmus University, Rotterdam; Karolinska Institutet; Stockholm County Council; Municipality of Rotterdam; Federal State of Mecklenburg-West Pomerania; AstraZeneca; GlaxoSmithKline; Siemens Healthcare; Novo Nordisk Foundation; Yrjö Jahnsson Foundation; Biomedicum Helsinki Foundation; Gyllenberg Foundation; Knut and Alice Wallenberg Foundation; Torsten and Ragnar Söderberg Foundation; Robert Dawson Evans Endowment, Boston University School of Medicine; Instrumentarium Science Foundation; Jenny and Antti Wihuri Foundation and the Canadian Primary Biliary Cirrhosis Society. A full list of acknowledgments is provided in the Supplementary Note.

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65Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts, USA
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67Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA
68Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
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75Institute of Diagnostics, Clinical Chemistry, University of Oulu, Oulu, Finland
76Department of Internal Medicine and Biocenter Oulu, Clinical Research Center, University of Oulu, Oulu, Finland
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91Klinikum Grosshadern, Munich, Germany
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98Oxford National Institute for Health Research Biomedical Research Centre, Churchill Hospital, Oxford, UK
99Institute of Epidemiology II, Helmholtz Zentrum M"unchen, German Research Center for Environmental Health, Neuherberg, Germany
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Fig 1

Summary of study design
Fig2

Manhattan plots of association of SNPs with ALT, ALP and GGT in the GWAS. SNPs reaching genome-wide significance ($P < 1 \times 10^{-8}$) are red; SNPs with $P > 1 \times 10^{-8}$ and $P < 1 \times 10^{-7}$ are green.
<table>
<thead>
<tr>
<th>Region</th>
<th>Sentinel SNP</th>
<th>Position</th>
<th>Genes of interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| 4q22   | rs6834314    | 88,432,832 | 3.1 × HSD17B1
|        |              |           | 3ne, MAPK10
| 8q24   | rs2954021    | 126,551,259 | 5.3 × TRIB1
|        |              |           | 1e
| 10q24^a| rs10883437   | 101,785,351 | 4.0 × CPN1
|        |              |           | 1e
| 22q13^a| rs738409     | 42,656,060  | 1.2 × PNPLA3
|        |              |           | 3ne, SAMM50
|        |              |           | 10e
| ALP    |              |          |                  |
| 1p36.12^a| rs1976403   | 21,639,040 | 1.8 × ALPL
|        |              |           | 1e, NBPF3
| 2q4    | rs16856332   | 169,548,820 | 1.6 × ABCB1
|        |              |           | 1ng
| 6p22^a | rs1883415    | 24,599,454  | 5.6 × ALDH5A1
|        |              |           | 1e, GPLD1
| 8p23   | rs6984305    | 9,215,678   | 2.1 × PPP1R3B
|        |              |           | 1e
| 8q24   | rs2954021    | 126,551,259 | 2.3 × TRIB1
|        |              |           | 1e
| 9q21   | rs10819937   | 103,263,054 | 1.0 × ALDOB
|        |              |           | 1e, C9orf125
| 9q34^a | rs579459     | 135,143,989 | 2.6 × ABO
|        |              |           | 1e
| 10q21^a| rs7923609    | 64,803,828  | 5.9 × IMJD1C
|        |              |           | 1e, NRBF2
| 11q12  | rs174601     | 61,379,716  | 2.6 × C11orf10
|        |              |           | 1e, FADS1
| 11q24  | rs2236653    | 125,788,995 | 1.8 × ST3GAL4
|        |              |           | 1e
| 16q22  | rs7186908    | 70,777,874  | 4.8 × HPR
|        |              |           | 1e, PMFBP1
| 17p13  | rs314253     | 7,032,374   | 8.4 × ASGR1
|        |              |           | 2e, DLG4
| 19q13^a| rs281377     | 53,898,415  | 1.1 × FUT2
|        |              |           | 1e
| 20p11  | rs7267979    | 25,246,087  | 7.4 × ABHD12
|        |              |           | 1e, GINS1

Table 1

Genetic loci associated with concentrations of liver enzymes in plasma at $P < 1 \times 10^{-8}$ in the GWAS
<table>
<thead>
<tr>
<th>Region</th>
<th>Sentinel SNP</th>
<th>Position</th>
<th>Alleles (R/E)</th>
<th>EAF</th>
<th>Effect (% confidence interval)</th>
<th>Genes of interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p36.13</td>
<td>rs1497406</td>
<td>16,377,907</td>
<td>A/G</td>
<td>0.56</td>
<td>3.8 (2.7–4.8)</td>
<td>$2.8 \times RSG1^e$, $EPHA2^{ne}$</td>
</tr>
<tr>
<td>1p22</td>
<td>rs12145922</td>
<td>88,918,822</td>
<td>C/A</td>
<td>0.61</td>
<td>2.8 (2.2–3.4)</td>
<td>$3.8 \times CCBL2^e$, $PKN2^a$</td>
</tr>
<tr>
<td>1p13</td>
<td>rs1335645</td>
<td>111,485,799</td>
<td>G/A</td>
<td>0.88</td>
<td>4.3 (3.5–5.2)</td>
<td>$7.3 \times CEPTI^{ne}$, $10^{-9}$ $DENND2D^e$</td>
</tr>
<tr>
<td>1q21</td>
<td>rs10908458</td>
<td>153,393,572</td>
<td>C/T</td>
<td>0.58</td>
<td>3.7 (3.1–4.2)</td>
<td>$1.7 \times DPM3^a$, $EFNA1^{ce}$, $PKLR^a$</td>
</tr>
<tr>
<td>2p23</td>
<td>rs1260326</td>
<td>27,584,444</td>
<td>C/T</td>
<td>0.38</td>
<td>3.2 (2.4–4.0)</td>
<td>$3.9 \times C2orf16^e$, $GCKR^{nc}$</td>
</tr>
<tr>
<td>2q12</td>
<td>rs13030978</td>
<td>191,825,483</td>
<td>C/T</td>
<td>0.32</td>
<td>3.7 (2.8–4.6)</td>
<td>$1.1 \times MYO1B^{ne}$, $STAT4^e$</td>
</tr>
<tr>
<td>2q37</td>
<td>rs2140773</td>
<td>233,221,419</td>
<td>C/A</td>
<td>0.61</td>
<td>2.9 (2.3–3.5)</td>
<td>$1.1 \times EFHD1^{ne}$</td>
</tr>
<tr>
<td>3q26</td>
<td>rs10513686</td>
<td>172,208,236</td>
<td>G/A</td>
<td>0.14</td>
<td>4.9 (4.0–5.7)</td>
<td>$6.1 \times SLC2A2^{nc}$</td>
</tr>
<tr>
<td>4q31</td>
<td>rs4547811</td>
<td>147,014,071</td>
<td>T/C</td>
<td>0.18</td>
<td>6.4 (5.0–7.9)</td>
<td>$2.5 \times ZNF827^a$</td>
</tr>
<tr>
<td>5p15</td>
<td>rs6888304</td>
<td>31,056,278</td>
<td>G/A</td>
<td>0.74</td>
<td>2.7 (2.0–3.5)</td>
<td>$1.2 \times CDH6^a$</td>
</tr>
<tr>
<td>5q11</td>
<td>rs4074793</td>
<td>52,228,882</td>
<td>A/G</td>
<td>0.07</td>
<td>5.5 (3.3–7.7)</td>
<td>$3.4 \times ITGA1^a$</td>
</tr>
<tr>
<td>6p12</td>
<td>rs9296736</td>
<td>54,032,656</td>
<td>C/T</td>
<td>0.31</td>
<td>3.0 (2.1–4.0)</td>
<td>$2.6 \times MLIP^{ne}$</td>
</tr>
<tr>
<td>7q11</td>
<td>rs17145750</td>
<td>72,664,314</td>
<td>T/C</td>
<td>0.86</td>
<td>4.5 (2.9–6.3)</td>
<td>$2.9 \times MLXIPL^{nce}$</td>
</tr>
<tr>
<td>10q23</td>
<td>rs754466</td>
<td>79,350,440</td>
<td>A/T</td>
<td>0.24</td>
<td>3.5 (2.2–4.8)</td>
<td>$6.4 \times DLG5^a$</td>
</tr>
<tr>
<td>12q24^a</td>
<td>rs7310409</td>
<td>119,909,244</td>
<td>A/G</td>
<td>0.59</td>
<td>6.8 (5.7–7.8)</td>
<td>$7.0 \times HNF1A^{nc}$, $C12orf27^{ne}$</td>
</tr>
<tr>
<td>14q32</td>
<td>rs944002</td>
<td>102,642,568</td>
<td>A/G</td>
<td>0.21</td>
<td>6.3 (4.9–7.7)</td>
<td>$5.8 \times C14orf73^{nc}$</td>
</tr>
<tr>
<td>15q21</td>
<td>rs339969</td>
<td>58,670,573</td>
<td>C/A</td>
<td>0.62</td>
<td>4.5 (3.9–5.1)</td>
<td>$6.6 \times RORA^a$</td>
</tr>
<tr>
<td>15q23</td>
<td>rs8038465</td>
<td>71,765,390</td>
<td>C/T</td>
<td>0.39</td>
<td>2.4 (1.8–3.0)</td>
<td>$1.4 \times CD276^{ne}$</td>
</tr>
<tr>
<td>16q23</td>
<td>rs4581712</td>
<td>79,055,102</td>
<td>C/A</td>
<td>0.27</td>
<td>3.2 (2.5–3.9)</td>
<td>$3.1 \times DYNLRB2^a$</td>
</tr>
<tr>
<td>17q24</td>
<td>rs9913711</td>
<td>67,609,756</td>
<td>G/C</td>
<td>0.65</td>
<td>2.4 (1.8–3.0)</td>
<td>$1.3 \times FLJ37644^e$, $SOX9^a$</td>
</tr>
<tr>
<td>18q21.31</td>
<td>rs12968116</td>
<td>53,473,500</td>
<td>T/C</td>
<td>0.87</td>
<td>4.8 (2.8–6.7)</td>
<td>$8.9 \times ATP8B1^{ncg}$</td>
</tr>
<tr>
<td>Region</td>
<td>Sentinel SNP</td>
<td>Position (bp)</td>
<td>Alleles (R/E)</td>
<td>EAF</td>
<td>Effect (%, 95% confidence interval)</td>
<td>P</td>
</tr>
<tr>
<td>------------</td>
<td>-------------</td>
<td>---------------</td>
<td>---------------</td>
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</tr>
<tr>
<td>18q21.32</td>
<td>rs4503880</td>
<td>54,235,034</td>
<td>C/T</td>
<td>0.21</td>
<td>3.6 (2.5–4.7)</td>
<td>3.0 × 10^{-12}</td>
</tr>
<tr>
<td>19q13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>rs516246</td>
<td>53,897,984</td>
<td>C/T</td>
<td>0.47</td>
<td>2.3 (1.8–2.9)</td>
<td>7.6 × 10^{-10}</td>
</tr>
<tr>
<td>22q11.21</td>
<td>rs1076540</td>
<td>16,819,958</td>
<td>T/C</td>
<td>0.78</td>
<td>4.8 (3.5–6.1)</td>
<td>9.6 × 10^{-17}</td>
</tr>
<tr>
<td>22q11.23</td>
<td>rs2739330</td>
<td>22,625,286</td>
<td>C/T</td>
<td>0.42</td>
<td>3.7 (2.7–4.6)</td>
<td>1.7 × 10^{-9}</td>
</tr>
<tr>
<td>22q11.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>rs2073398</td>
<td>23,329,104</td>
<td>C/G</td>
<td>0.34</td>
<td>12.3 (10.9–13.7)</td>
<td>1.1 × 10^{-10}</td>
</tr>
</tbody>
</table>

Alleles are given as the reference (R) allele/effect (E). EAF, effect allele frequency; effect is change in concentration of liver enzyme in plasma per copy of effect allele.

<sup>a</sup>Previously reported associations. Annotation for genes of interest:
<sup>n</sup>nearest;
<sup>e</sup>expression QTL;
<sup>c</sup>coding SNP;
<sup>g</sup>GRAIL;
<sup>o</sup>known biology.
Association of FADS1, FADS2, GCKR, HNF1A, TRIB1 and PPP1R3B loci with NMR metabolome. Bars are for $-\log_{10}$ P value, signed for direction of effect.