# Genome-wide association study of circulating liver enzymes reveals an expanded role for manganese transporter SLC30A10 in liver health

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## Abstract

To better understand molecular pathways underlying liver health and disease, we performed genome-wide association studies (GWAS) on circulating levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) across 408,300 subjects from four ethnic groups in the UK Biobank, focusing on variants associating with both enzymes. Of these variants, the strongest effect is a rare (MAF in White British = 0.12%) missense variant in the gene encoding manganese efflux transporter SLC30A10, Thr95Ile (rs188273166), associating with a 5.9% increase in ALT and a 4.2% increase in AST. Carriers have higher prevalence of all-cause liver disease (OR = 1.70; 95% CI = 1.24 to 2.34) and higher prevalence of extrahepatic bile duct cancer (OR = 23.8; 95% CI = 9.1 to 62.1) compared to non-carriers. Over 4% of the cases of extrahepatic cholangiocarcinoma in the UK Biobank carry *SLC30A10* Thr95Ile. Unlike variants in *SLC30A10* known to cause the recessive syndrome hypermanganesemia with dystonia-1 (HMNDYT1), the Thr95Ile variant has a detectable effect even in the heterozygous state. Also unlike HMNDYT1-causing variants, Thr95Ile results in a protein that is properly trafficked to the plasma membrane when expressed in HeLa cells. These results suggest that coding variation in *SLC30A10* impacts liver health in more individuals than the small population of HMNDYT1 patients.


## Introduction

Liver disease remains an area of high unmet medical need, and better characterizing the environmental and genetic determinants of liver disease is key to developing new therapeutic strategies. In addition, liver injury is a common side effect of drugs, and is a frequent reason that drugs fail to progress through the development pipeline; understanding the molecular mechanisms of liver injury can aid in rational drug design to avoid off-target effects. Circulating liver enzymes

44 are sensitive biomarkers of liver injury; in particular, alanine aminotransferase (ALT) and aspartate
45 aminotransferase (AST) are released into the circulation during damage to hepatocyte
46 membranes[1,2]. One powerful approach for understanding the molecular basis of liver disease has
47 been to perform genome-wide association studies (GWAS) of levels of circulating liver enzymes
48 across large population samples[1,3-13]. Combined GWAS of ALT and AST have previously revealed
49 genetic associations providing potential therapeutic targets for liver disease such as *PNPLA3*[14] and
50 *HSD17B13*[15]. To further study the genetics of hepatocellular damage, we performed GWAS on
51 circulating levels of ALT and AST in 408,300 subjects, meta-analyzed across four ethnic groups
52 in the UK Biobank.

53 # Results

54 ## GWAS summary

55 We performed a GWAS of ALT and AST in four sub-populations in the UK Biobank (Asian or
56 Asian British, Black or Black British, Chinese, and Black or Black British; sample sizes, number
57 of variants tested, and $\lambda_{GC}$ values, **Supplementary Table 1;** genome-wide significant associations,
58 **Supplementary Table 2**; Manhattan and QQ plots for each enzyme and sub-population,
59 **Supplementary Figures 1 and 2**). After meta-analyzing across sub-populations to obtain a single
60 set of genome-wide p-values for each enzyme (Manhattan plots, **Figure 1**), we found 244 and 277
61 independent loci associating at $p < 5 \times 10^{-8}$ with ALT and AST, respectively, defined by lead SNPs
62 separated by at least 500 kilobases and pairwise linkage disequilibrium (LD) $r^2$ less than 0.2.
63 Enzyme levels were strongly associated with coding variants in the genes encoding the enzymes,
64 representing strong protein quantitative trait loci in *cis* (cis-pQTLs). For example rs147998249, a
65 missense variant Val452Leu in *GPT* (glutamic-pyruvic transaminase) encoding ALT, strongly

66 associates with ALT ($p < 10^{-300}$) and rs11076256, a missense variant Gly188Ser in *GOT2*
67 (glutamic-oxaloacetic transaminase 2) encoding the mitochondrial isoform of AST, strongly
68 associates with AST ($p = 6.3 \times 10^{-62}$). While these strong *cis*-pQTL effects validated our ability to
69 detect direct genetic influences on ALT and AST levels, the aim of this study was to detect genetic
70 determinants of liver health that in turn had downstream effects on both ALT and AST due to
71 hepatocellular damage; therefore we focused the remainder of our analyses on the variants only
72 associated with levels of both enzymes (labeled with black text on **Figure 1**).

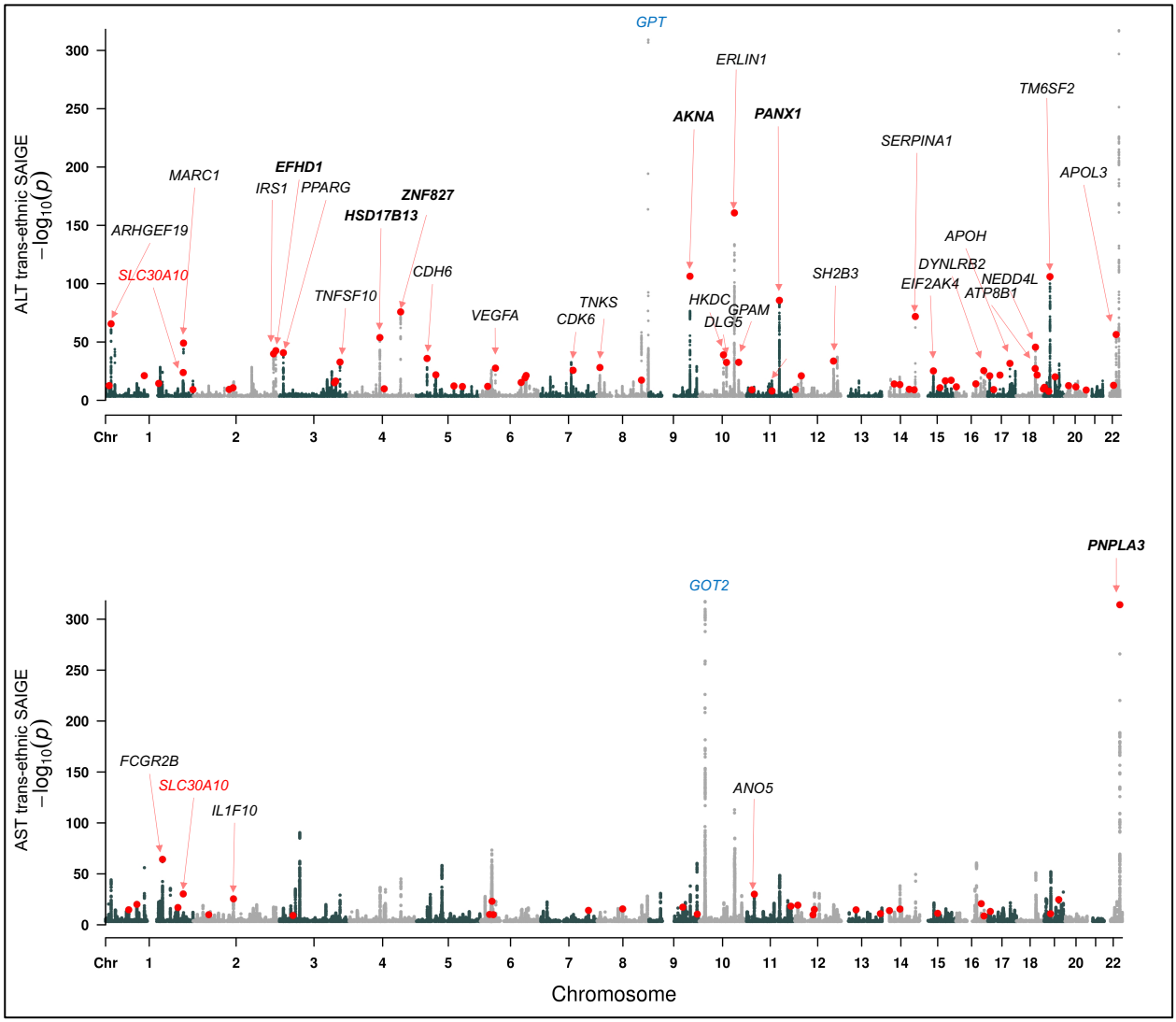


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**Figure 1: Manhattan plots showing trans-ethnic GWAS results for ALT and AST**. Red dots indicate lead SNPs for shared signals between the two GWAS; for clarity, the shared signals are marked only once, on the plot for the GWAS in which the more significant association is detected. Cis-pQTLs (at *GPT* and *GOT2*) are labeled in blue. Loci with shared signals are labeled (for clarity, only when $p < 10\text{-}25$ and only on the GWAS for which the association is most significant). Loci previously reported to associate with both ALT and AST are named in bold. *SLC30A10*, the main topic of this report, is labeled in red on both plots.

Focusing only on loci with both ALT and AST GWAS signals (lead SNPs from either GWAS were identical or shared proxies with $r^2 \geq 0.8$), we found a total of 100 independent loci associated with both enzymes (**Figure 2, Supplementary Table 3**). As expected, effect sizes on ALT and AST at these loci were highly correlated ($r = 0.98$), and at all 100 loci the direction of effect on ALT and AST was concordant. Of these 100 loci, six were coincident or in strong LD with a published ALT or AST SNP in the EBI-NHGRI GWAS Catalog, and 15 were within 500kb of a published ALT or AST SNP; 33 of the loci harbored a moderate or high-impact variant as defined by the VEP or LOFTEE algorithms; and of the remaining 67 entirely noncoding loci, 19 were coincident or in strong LD with the strongest eQTL for a gene in liver, muscle, or kidney suggesting that effects on gene expression may drive their associations with ALT and AST. A majority (70 of the 100 loci) were shared with a distinct published association in the GWAS Catalog, suggesting pleiotropy with other traits.

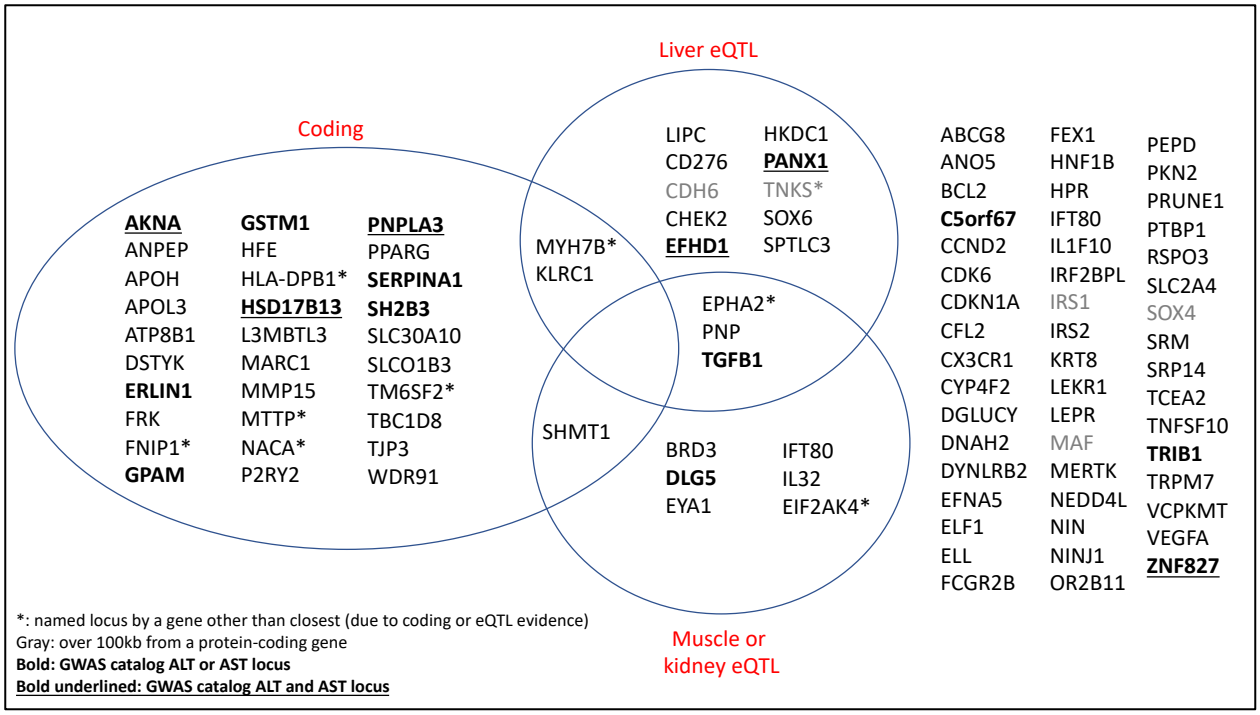


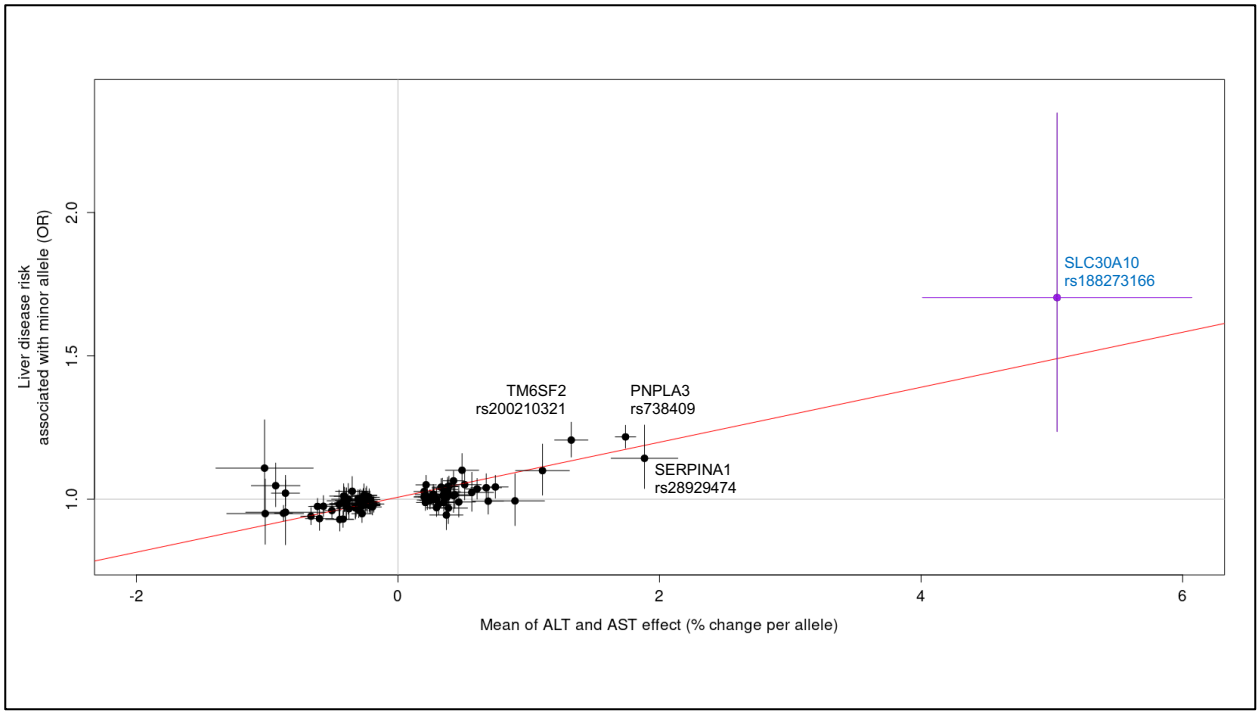

**Figure 2: Classification of the top ALT- and AST- associated loci based on annotations**. Unless otherwise noted with an asterisk, loci are named by the closest protein-coding gene. "Coding" indicates that one of the variants linked to the lead SNP is predicted to have a moderate or high impact on a protein-coding gene. "Liver eQTL" and "Muscle or kidney eQTL" indicate that one of the variants linked to the lead SNP is the strongest eQTL for a gene in those tissues by GTEx.

The strongest estimated effect of the lead SNPs on either enzyme was a novel association: rs188273166, a rare (MAF in White British = 0.12%) missense variant (Thr95Ile) in *SLC30A10*, associated with a 4.2% increase in ALT (95% CI, 4.6% to 7.1%; $p = 1.6 \times 10^{-24}$) and 5.9% increase in AST (95% CI: 3.4% to 5.0%; $p = 4.9 \times 10^{-31}$). Because Thr95Ile is coding and not strongly linked to any other SNPs, we considered it likely to be the causal variant driving the association at the *SLC30A10* locus. *SLC30A10* encodes a manganese efflux transporter (solute carrier family 30 member 10, also known as zinc transporter 10 or ZnT10)[16,17]. Loss-of-function mutations in *SLC30A10* have been reported to cause a rare recessive syndrome, hypermanganesemia with

109 dystonia 1 (HMNDYT1), characterized by cirrhosis, dystonia, parkinsonism, polycythemia, and
110 hypermanganesemia[17,19-24]. The next strongest effect on either enzyme was rs28929474, a
111 missense variant (the Pi-Z allele) in *SERPINA1* (serpin family A member 1) which causes alpha-
112 1 antitrypsin deficiency (AATD) in its homozygous state[18], associated with a 2.5% increase in
113 ALT (95% CI, 2.1% to 2.8%; $p = 1.4 \times 10^{-72}$) and 1.3% increase in AST (95% CI, 1.1% to 1.5%;
114 $p = 3.2 \times 10^{-50}$); AATD manifests with both lung and liver damage. The most statistically
115 significant association with either enzyme was rs738409, a common missense variant (Ile148Met)
116 in *PNPLA3* (patatin like phospholipase domain containing 3) known to strongly increase risk of
117 liver disease[14]; it is associated with a 2.2% increase in ALT (95% CI, 2.1% to 2.3%; $p < 10^{-300}$)
118 and a 1.3% increase in AST (95% CI, 1.3% to 1.4%; $p < 10^{-300}$).
119

120 ## Association of ALT- and AST-associated variants with liver disease risk

121 We tested the 100 lead SNPs from the ALT and AST GWAS analysis for association with a
122 combined liver disease phenotype (ICD10 codes K70-77, I85, and C22; 14,648 cases and 487,968
123 controls across the entire biobank), meta-analyzing liver disease association results across all four
124 sub-populations (**Supplementary Table 3)**. Of the 100 lead SNPs, 26 SNPs associate with liver
125 disease with $p < 0.05$. As expected, variants associated with an increase in ALT and AST tend to
126 be associated with a proportional increase in liver disease risk (across all lead SNPs, effect size r
127 = 0.73 for ALT, r = 0.71 for AST; **Figure 3**). Consistent with *SLC30A10* Thr95Ile (rs188273166)
128 having the strongest effect on ALT and AST of all lead SNPs, it also has the strongest estimated
129 effect on liver disease risk (OR = 1.70; 95% CI, 1.24 to 2.34; $p = 7.4 \times 10^{-3}$).
130

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132 **Figure 3: Comparison of effect of lead SNPs on ALT and AST with effect on liver disease**. Effect sizes (beta in
133 units of $\log_{10}$ ALT and AST and odds ratios) are from PLINK analysis; error bars are 95% confidence intervals on
134 these effect sizes.

135

136 Because *SLC30A10* Thr95Ile had the strongest effect of all of our lead SNPs and also has not been
137 reported as being associated with any phenotypes in the literature, we centered the following
138 analyses on better understanding its function.

139

## 140 Validation of *SLC30A10* Thr95Ile genotype and replication of ALT and AST
## 141 associations

142 Because rare variants are especially prone to errors in array genotyping[25], we sought to validate
143 the array genotype calls for *SLC30A10* Thr95Ile in a subset of 301,473 individuals who had also
144 been exome sequenced (**Supplementary Table 4**). The one individual homozygous for the minor
145 (alternate) allele by array was confirmed by exome sequencing; no further homozygotes were

146 identified. Of 702 individuals called as heterozygous for Thr95Ile by array data who had available
147 exome data, 699 (99.6%) were confirmed heterozygous by exome sequencing.
148
149 To replicate the rs188273166 association with ALT and AST in an independent cohort, we
150 identified ethnic groups besides the White British subpopulation harboring the variant. The only
151 two other populations with a substantial number of *SLC30A10* Thr95Ile carriers were individuals
152 identifying as Other White (33/16,299 are carriers) and as White Irish (35/12,715 are carriers)
153 (**Supplementary Table 5**). Meta-analyzing the association results across these two outgroups
154 revealed higher mean ALT and AST in the small sample of additional Thr95Ile carriers, although
155 the effect was not statistically significant for ALT (ALT increase = 3.7%; 95% CI, -1.1% to 8.4%,
156 p = 0.084; AST increase = 4.1%; 95% CI, 1.1% to 7.2%; $p = 1.7 \times 10^{-3}$) (**Supplementary Figure**
157 **3**).
158
159

160 ## Magnitude of ALT and AST elevation in *SLC30A10* Thr95Ile carriers

161 After establishing the association between *SLC30A10* Thr95Ile and ALT and AST, we sought to
162 further explore the relationship between genotype and enzyme levels to understand clinical
163 relevance. Carriers of Thr95Ile had a mean ALT of 27.37 U/L vs 23.54 U/L for noncarriers, and a
164 mean AST of 28.85 U/L vs 26.22 U/L for noncarriers. Counting individuals with both ALT and
165 AST elevated above 40 U/L, a commonly-used value for the upper limit of normal (ULN)[2], 5.6%
166 of carriers vs 3.6% of noncarriers had both enzymes elevated at the time of their UK Biobank
167 sample collection, a relative increased risk of 58% (**Supplementary Table 6**).
168

169 ## Independence of *SLC30A10* Thr95Ile from neighboring ALT and AST associations

170 Because we applied distance and LD pruning to the results of the genome-wide scan, it was unclear
171 how many independent association signals existed at the *SLC30A10* locus. Revisiting trans-ethnic
172 association results in a window including 1 Mb flanking sequence upstream and downstream of
173 *SLC30A10* revealed 76 SNPs with genome-wide significant associations with both ALT and AST
174 (**Figure 4**). These 76 SNPs clustered into three loci: *SLC30A10* (only Thr95Ile, rs188273166);
175 *MARC1* (mitochondrial amidoxime reducing component 1, lead SNP rs2642438 encoding
176 missense Ala165Thr, reported in a recent preprint[26], and six additional SNPs together spanning 68
177 kilobases); and *LYPLAL1-ZC3H11B* (intergenic region between lyophospholipase like 1 and zinc
178 finger CCCH-type containing 11B, with array-genotyped SNP rs6541227 and 67 imputed SNPs
179 spanning 46 kilobases), a locus previously reported to associate with non-alcoholic fatty liver
180 disease (NAFLD)[27].
181

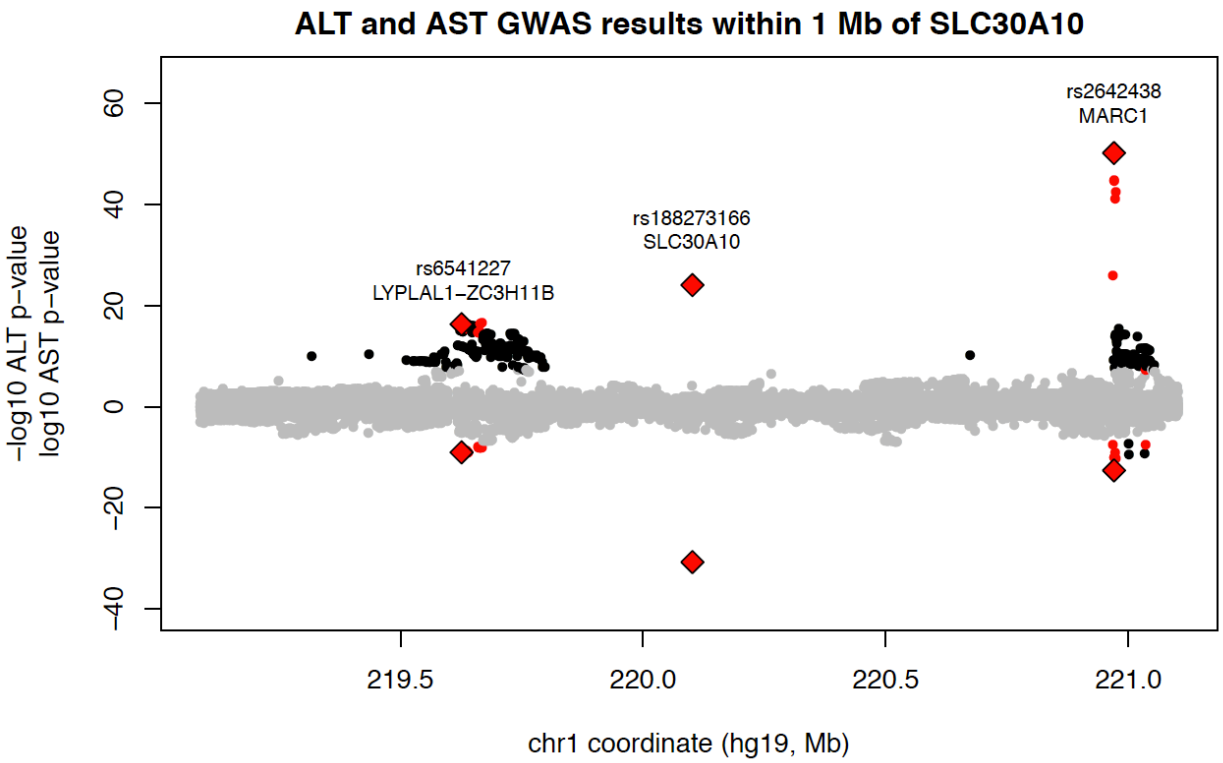



**Figure 4: Miami plot of trans-ethnic SAIGE GWAS results within 1 Mb of the gene body of *SLC30A10*.** ALT associations are shown in the positive direction and AST associations in the negative direction. SNP associations reaching genome-wide significance for one enzyme are colored black; for both enzymes, colored red; directly-genotyped SNPs significant for both enzymes, red diamonds.

To test for independence between these three loci, we performed ALT and AST association tests for each of the three array-typed SNPs while including the genotype of either one or both of the others as covariates. Associations were similar in these conditional analyses, suggesting that each of these three associations are not confounded by linkage disequilibrium with the other regional association signals (**Supplementary Table 7**.) Therefore *SLC30A10* Thr195Ile has a novel independent association with ALT and AST levels.

195 ## Linkage of Thr95Ile to GWAS SNPs at *SLC30A10*

196 A GWAS of circulating toxic metals[28] discovered an association between a common intronic SNP
197 in *SLC30A10* (rs1776029; MAF in White British, 19.5%) and blood manganese levels, where the
198 reference allele – which is the minor allele – is associated with increased circulating manganese.
199 We calculated linkage disequilibrium statistics between rs1776029 and Thr95Ile and found that
200 the minor allele of Thr95Ile (A) was in almost perfect linkage with the minor allele of rs1776029
201 (A) (r2 = 0.005, D' = 0.98); Thr95Ile (rs188273166) is 154 times more frequent among carriers of
202 at least one copy of the minor allele of common SNP rs1776029 (95% CI = 84 – 325; Fisher's $p <$
203 $2.2 \times 10^{-16}$). These results suggest that the previously reported association of rs1776029 with
204 circulating manganese may be partially or completely explained by linkage with Thr95Ile
205 (**Supplementary Table 8**); however genotypes of Thr95Ile in the manganese GWAS or
206 manganese measurements in the UK Biobank would be needed in order to perform conditional
207 analysis. We then systematically tested nearby SNPs reported in the GWAS Catalog for any
208 phenotype for linkage to Thr95Ile, measured by high |D'|. Combining GWAS Catalog information
209 and |D'| calculations, we find nearly perfect linkage (|D'| > 0.90) between rs188273166-A (rare
210 missense Thr95Ile) with rs1776029-A (intronic), rs2275707-C (3'UTR), and rs884127-G
211 (intronic), all within the gene body of *SLC30A10* (**Supplementary Table 9**). In addition to
212 increased blood Mn[28], these three common alleles have been associated with decreased
213 magnesium/calcium ratio in urine[29], decreased mean corpuscular hemoglobin (MCH)[30-32],
214 increased red blood cell distribution width[30-32], and increased heel bone mineral density (BMD)[32-
215 35].
216

217 A recent study not yet in the GWAS catalog reported an association between another common
218 intronic SNP in *SLC30A10* (rs759359281; MAF in White British, 5.6%) and liver MRI-derived
219 cT1 measures, a proxy for liver fibrosis and steatohepatitis[36]. However, the reported cT1-
220 increasing allele (liver disease risk allele) of rs759359281, which is the minor allele, is in complete
221 linkage (D' = 1) with the major allele of Thr95Ile (rs188273166); in other words, the cT1-
222 increasing allele and Thr95Ile liver disease risk allele occur on different haplotypes, suggesting
223 that the mechanism of this reported cT1 association is independent of Thr95Ile.
224

225 ## Phenome-wide associations of *SLC30A10* Thr95Ile

226 To explore other phenotypes associated with *SLC30A10* Thr95Ile, we tested for association with
227 135 quantitative traits and 4,533 ICD10 diagnosis codes within the White British population
228 (**Supplementary Tables 10 and 11**). Besides ALT and AST elevation, rs188273166 was
229 associated with other indicators of hepatobiliary damage such as decreased HDL cholesterol and
230 apolipoprotein A (ApoA)[37], decreased albumin, and increased GGT. Other phenome-wide
231 significant quantitative trait associations were increases in hemoglobin concentration and
232 hematocrit (**Table 1**).
233

| Quantitative trait | Association with *SLC30A10* Thr95Ile | | | | | |
|---|---|---|---|---|---|---|
| | P | signif. | Effect (SD) | 95% CI lower | 95% CI upper | N |
| Aspartate aminotransferase | 2.90E-32 | *** | 0.38 | 0.31 | 0.44 | 387770 |
| Alanine aminotransferase | 1.95E-25 | *** | 0.32 | 0.25 | 0.38 | 389063 |
| HDL cholesterol | 1.18E-15 | *** | -0.22 | -0.28 | -0.15 | 356214 |
| Apolipoprotein A | 7.25E-11 | *** | -0.19 | -0.25 | -0.12 | 354247 |
| Albumin | 2.12E-08 | *** | -0.17 | -0.24 | -0.10 | 356374 |
| Hemoglobin concentration | 8.11E-06 | ** | 0.09 | 0.04 | 0.15 | 396065 |
| Hematocrit (percentage) | 1.33E-05 | ** | 0.10 | 0.04 | 0.15 | 396065 |
| Gamma glutamyltransferase | 9.86E-05 | ** | 0.12 | 0.05 | 0.18 | 388997 |
| Heel BMD | 2.05E-03 | * | 0.13 | 0.05 | 0.22 | 237053 |

| | | | | | | |
|---|---|---|---|---|---|---|
| MCHC | 7.73E-02 | N.S. | 0.05 | -0.01 | 0.12 | 327167 |
| Erythrocyte dist. width | 3.46E-01 | N.S. | 0.02 | -0.04 | 0.09 | 327168 |

**Table 1: Association results of *SLC30A10* Thr95Ile with selected quantitative traits.** P values are from SAIGE analysis; effect size estimates are from PLINK analysis. (***) indicates genome-wide significance $p < 5 \times 10^{-8}$; (**) indicates phenome-wide significance of $p < 3.7 \times 10^{-4}$; (*) indicates nominal significance of $p < 0.05$. All traits are rank-based inverse-normal transformed and effect size is in units of standard deviations of the transformed values.

The only phenome-wide significant association of a diagnosis with *SLC30A10* Thr95Ile was C24.0, extrahepatic bile duct carcinoma (OR = 23.8; 95% CI = 9.1 to 62.1; $p = 1.2 \times 10^{-6}$). The overall prevalence of extrahepatic bile duct in the UK Biobank is 119 in 502,616 individuals (1 in 4,224) while the prevalence among carriers is 5 in 1,171 (1 in 234); 4% of individuals with extrahepatic bile duct carcinoma carry Thr95Ile. As there is an overlap of extrahepatic bile duct carcinoma cases with intrahepatic bile duct carcinoma cases, which are included in the all-cause liver disease phenotype, we examined whether the association of Thr95Ile with all-cause liver disease (noted earlier) was driven solely by individuals with bile duct carcinoma. However, we found in this phenome-wide scan that the ICD10 code block K70-K77, encompassing non-cancer liver disease, was also associated with *SLC30A10* Thr95Ile (OR = 1.6; 95% CI = 1.2 to 2.3; p = 0.048). This suggests that the presence of Thr95Ile affects both bile duct carcinoma and liver disease risk. Searching for neurological manifestations similar to HMNDYT1, we find no association with Parkinson's disease or dystonia but note that we are powered to exclude only strong effects because of the small case number for these traits; our confidence interval for association with Parkinson's and dystonia both include an effect of the same magnitude as on liver disease (**Table 2**).

| | Association with *SLC30A10* Thr95Ile | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Diagnosis | P | sig. | N cases (biobank) | Prevalence (biobank) | N cases (carriers) | Prevalence (carriers) | OR | 95% CI |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| C24.0: Malignant neoplasm of extrahepatic bile duct | 1.17E-06 | ** | 119 | 0.02% | 5 | 0.45% | 23.8 | 9.1 | 62.1 |
| K70-K77: Diseases of liver | 4.78E-02 | * | 13884 | 2.76% | 40 | 3.58% | 1.6 | 1.2 | 2.3 |
| D50: Iron deficiency anemia | 1.35E-02 | * | 22371 | 4.45% | 62 | 5.55% | 1.4 | 1.0 | 1.8 |
| G20-G26: Extrapyramidal and movement disorders | 7.64E-01 | N.S. | 10160 | 2.02% | 19 | 1.70% | 0.8 | 0.5 | 1.3 |
| G20: Parkinson's disease | 3.95E-01 | N.S. | 2426 | 0.48% | 4 | 0.36% | 0.5 | 0.1 | 1.9 |
| G21: Secondary parkinsonism | 2.77E-01 | N.S. | 812 | 0.16% | 0 | NA | 1.6 | 0.3 | 10.6 |
| G24: Dystonia | 5.69E-01 | N.S. | 2354 | 0.47% | 4 | 0.36% | 1.2 | 0.5 | 3.0 |

**Table 2: association results of *SLC30A10* Thr95Ile with selected ICD10 diagnosis codes.** P values are from SAIGE analysis; effect size estimates are from PLINK analysis (exponentiated betas from logistic regression). (**) indicates phenome-wide significance of $p < 1.1 \times 10^{-5}$; (*) indicates nominal significance of $p < 0.05$.

To test whether the association with extrahepatic bile duct cancer was driven by a nearby association, we performed an association study of the diagnosis with variants within a window including 1 Mb flanking sequence upstream and downstream of *SLC30A10*; Thr95Ile was the strongest association (**Supplementary Figure 4**).

## Bioinformatic characterization of *SLC30A10* Thr95Ile

To understand potential functional mechanisms of the Thr95Ile variant, we examined bioinformatic annotations of SLC30A10 Thr85Ile. The UNIPROT database shows that Thr95Ile occurs in the third of six transmembrane domains and shares a domain with a variant known to cause HMNDYT1 (**Figure 5**). In silico analysis predicts that Thr95Ile is a damaging mutation; it has a CADD PHRED score of 23.9, placing it in the top 1% of deleteriousness scores for genome wide potential variants; the algorithm SIFT predicts it as deleterious; and the algorithm PolyPhen-2 gives it a HumDiv score of 0.996 (probably damaging) and a HumVar score of 0.900 (possibly damaging.) Cross-species protein sequence alignment in PolyPhen-2 shows only threonine or

serine at position 95 across animals. These properties suggest that Thr95Ile substitution ought to have an effect the function of the SLC30A10 protein.

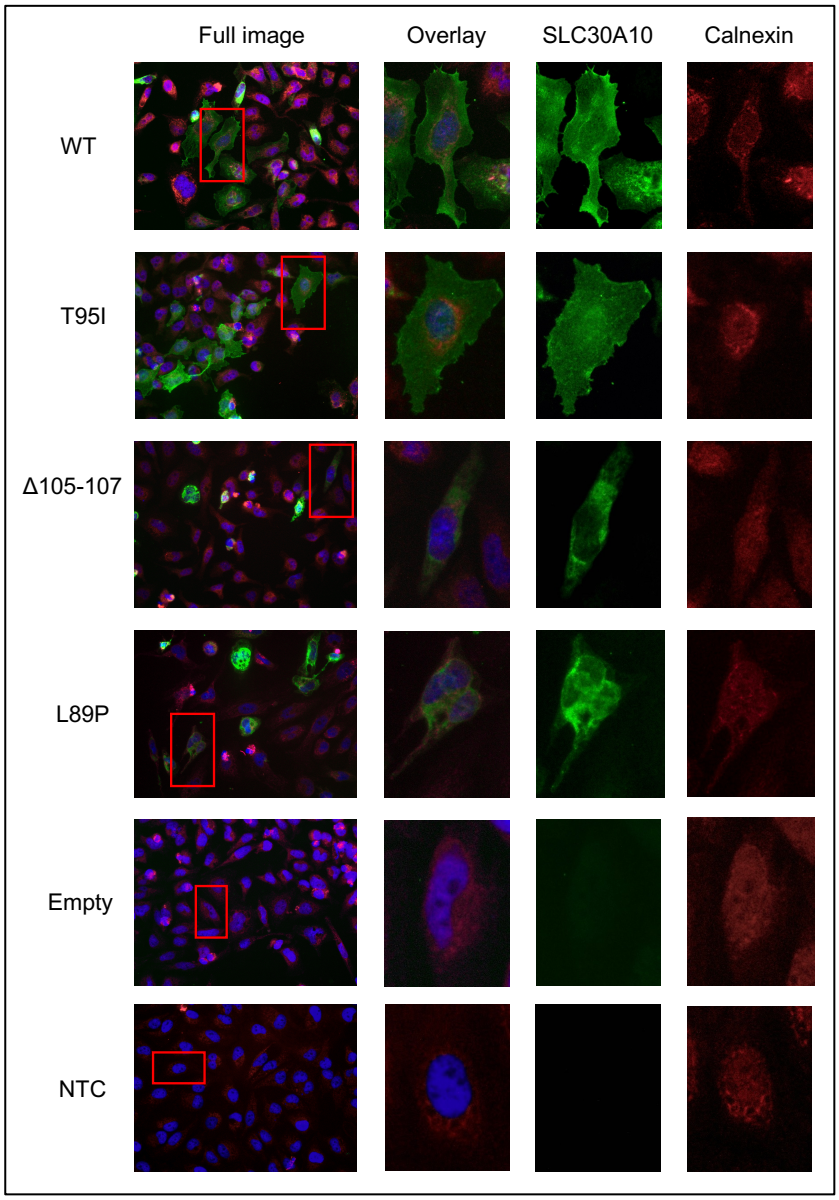
```
        10         20         30         40         50
MGRYSGKTCR LLFMLVLTVA FFVAELVSGY LGNSIALLSD SFNMLSDLIS
        60         70         80         90        100
LCVGLSAGYI ARRPTRGFSA TYGYARAEVV GALSNAVFLT ALCFTIFVEA
       110        120        130        140        150
VLRLARPERI DDPELVLIVG VLGLLVNVVG LLIFQDCAAW FACCLRGRSR
       160        170        180        190        200
RLQQRQQLAE GCVPGAFGGP QGAEDPRRAA DPTAPGSDSA VTLRGTSVER
       210        220        230        240        250
KREKGATVFA NVAGDSFNTQ NEPEDMMKKE KKSEALNIRG VLLHVMGDAL
       260        270        280        290        300
GSVVVVITAI IFYVLPLKSE DPCNWQCYID PSLTVLMVII ILSSAFPLIK
       310        320        330        340        350
ETAAILLQMV PKGVNMEELM SKLSAVPGIS SVHEVHIWEL VSGKIIATLH
       360        370        380        390        400
IKYPKDRGYQ DASTKIREIF HHAGIHNVTI QFENVDLKEP LEQKDLLLLC
       410        420        430        440        450
NSPCISKGCA KQLCCPPGAL PLAHVNGCAE HNGGPSLDTY GSDGLSRRDA
       460        470        480
REVAIEVSLD SCLSDHGQSL NKTQEDQCYV NRTHF
```

**Figure 5: Protein sequence of SLC30A10 along with experimental evidence cited by UNIPROT.** Underlined are the six transmembrane domains. Highlighted red is Thr95Ile. Highlighted in yellow are variants that demonstrated abolished Mn transport or membrane localization in vitro. Highlighted in blue are variants that demonstrated lesser or no effect on Mn transport or membrane localization in vitro. In bold are variants known to cause SLC30A10 deficiency (HMNDYT1).

## Characterization of *SLC30A10* variants *in vitro*

To test the protein localization of SLC30A10 harboring Thr95Ile as well as other variants, we created constructs with Thr95Ile (rs188273166) and the HMNDYT1-causing variants Leu89Pro (rs281860284) and del105-107 and transfected these constructs into HeLa cells.

289 Immunofluorescence staining revealed membrane localization for wild-type (WT) SLC30A10
290 which was abolished by the two HMNDYT1 variants, consistent with previous reports which
291 showed that the HMNDYT1 variants proteins are mislocalized in the endoplasmic reticulum
292 (ER)[38]. In contrast, Thr95Ile showed membrane localization similar to WT, suggesting that
293 Thr95Ile does not cause a deficit in protein trafficking to the membrane (**Figure 6**). Thr95Ile may
294 affect SLC30A10 function in other ways, for example altering Mn transport efficiency.




295

296 **Figure 6: Immunofluorescence imaging of SLC30A10 protein constructs expressed in cultured HeLa cells.** WT
297 = wild type; T95I = Thr95Ile; Δ105-107 and L89P, HMNDYT1-causing variants reported previously [38]; empty =
298 transfected with empty vector; NTC = non transfected control. Calnexin staining (in red) indicates the endoplasmic
299 reticulum (ER).

# 300 Discussion

## 301 Expanded genetic landscape of risk for hepatocellular damage

302 Our trans-ethnic GWAS of ALT and AST reveals a broad genetic landscape of loci that modulate
303 risk of hepatocellular damage or other diseases that cause increases in circulating ALT and AST,
304 bringing the number of loci known to associate with levels for both enzymes from 10 (currently in
305 the GWAS Catalog) to 100. Two loci had been previously reported in majority-European ancestry
306 GWAS as associating with both ALT and AST: *PNPLA3*[3,4,7-9,39-41] and *HSD17B13*[3,7,15]; we detect
307 colocalized ALT and AST signals at both of these loci. Broadening beyond majority-European
308 ancestry GWAS, an additional eight loci have been previously identified: *PANX1*[7], *ALDH2*[6,8],
309 *CYP2A6*[7,39], *ABCB11*[7], *ZNF827*[7], *EFHD1*[7], *AGER-NOTCH4*[7,42], and *AKNA*[7]; we replicate four of
310 these in our trans-ethnic GWAS (*PANX1, ZNF827*, *EFHD1*, and *AKNA*.) We are limited by the
311 lack of diversity in the UK Biobank and expect that studies in more diverse populations will result
312 in the discovery of new loci and alleles.
313
314 Among the novel loci are many that had been previously identified as risk loci for liver disease,
315 but had never been explicitly associated through ALT or AST GWAS, such as *SERPINA1*
316 (associated with alpha-1 antitrypsin deficiency[18]), *HFE* (homeostatic iron regulator, associated
317 with hemochromatosis[43]), and *TM6SF2* (transmembrane 6 superfamily member 2, associated with
318 NAFLD[44-46]). Others are known to associate with biliary disease, such as *ABCG8* (ATP-binding

319 cassette subfamily G member 8, associated with gallstones[47]), *ANPEP* (alanyl aminopeptidase,
320 associated with gallstones[48]), *HNF1B* (HNF1 homeobox B, associated with gallstones[48]), and
321 *SLCO1B3* (solute carrier organic anion transporter family member 1B3, implicated in digenic rotor
322 type hyperbilirubinemia[49]), along with loci previously associated with increased GGT (*EPHA2*,
323 *CDH6*, *DLG5*, *CD276*, *DYNLRB2,* and *NEDD4L*)[3,7]. Consistent with the fact that ALT and AST
324 elevation can be caused by kidney or muscle damage, we detect an association with *ANO5*
325 (anoctamin 5), which has been implicated in several autosomal recessive muscular dystrophy
326 syndromes[50,51], and several loci associated with expression of genes in muscle or kidney but not
327 liver (*SHMT1, BRD3, DLG5, EYA1, IFT80, IL32*, and *EIF2AK4.*) We expect only a subset of the
328 loci from this screen to be directly causally implicated in hepatocellular damage; many may
329 predispose to a condition where liver damage is secondary or affect kidney or muscle, an important
330 limitation of this approach.
331

332 ## Properties of *SLC30A10* Thr95Ile

333 The variant with the strongest predicted effect on ALT and AST, *SLC30A10* Thr95Ile
334 (rs188273166), is a rare variant carried by 1,117 of the 487,327 array-genotyped participants in
335 the UK Biobank. While Thr95Ile is found in some individuals of non-European ancestry, it is at
336 much higher frequency in European-ancestry populations, with carrier frequency in our sample by
337 UK country of birth ranging from a minimum of 1 in 479 people born in Wales to a maximum of
338 1 in 276 people born in Scotland (**Supplementary Table 5**). The increased frequency we see in
339 European-ancestry populations is not merely due to those populations' overrepresentation in the
340 UK Biobank, but is also consistent with global allele frequency data catalogued in dbSNP[52].
341

342 The Thr95Ile variant occurs in the third of six transmembrane domains of the SLC30A10 protein[53],
343 the same domain affected by a previously-reported loss-of-function variant causing HMNDY1
344 (hypermanganesemia with dystonia 1), Leu89Pro (rs281860284)[38] (**Figure 6**). In vitro, Leu89Pro
345 abolishes trafficking of SLC30A10 to the membrane[38], and another study pointed to a functional
346 role of polar or charged residues in the transmembrane domains of SLC30A10 for manganese
347 transport function[54]. Bioinformatic analysis suggest that Thr95Ile should impact protein function.
348 Our site-directed mutagenesis experiment of SLC30A10 shows that Thr95Ile, unlike reported
349 HMNDYT1-causing variants, results in a protein that is properly trafficked to the cell membrane.
350 Further biochemical studies will be required to investigate whether the Thr95Ile variant of
351 SLC30A10 has reduced manganese efflux activity, which would be consistent with our genetic
352 observations.
353

354 ## Comparison of *SLC30A10* Thr95Ile phenotypes to HMNDYT1 phenotypes

355 *SLC30A10* (also known as *ZNT10*, and initially identified through sequence homology to zinc
356 transporters[16]) encodes a cation diffusion facilitator expressed in hepatocytes, the bile duct
357 epithelium, enterocytes, and neurons[22] that is essential for excretion of manganese from the liver
358 into the bile and intestine[17,22]. Homozygous loss-of-function of *SLC30A10* was recently identified
359 as the cause of the rare disease HMNDYT1, which in addition to hypermanganesemia and dystonia
360 is characterized by liver cirrhosis, polycythemia, and Mn deposition in the brain[19-24,38]. Other
361 hallmarks include iron depletion and hyperbilirubinemia. Mendelian disorders of *SLC30A10* and
362 the other hepatic Mn transporter genes *SLC39A8* (solute carrier family 39 member 8, causing
363 congenital disorder of glycosylation type IIn)[55] and *SLC39A14* (solute carrier family 39 member
364 14, implicated in hypermanganesemia with dystonia 2)[56], along with experiments in transgenic

365 mice [57,58], have confirmed the critical role of each of these genes in maintaining whole-body
366 manganese homeostasis[59]. Notably, while all three of the genes have Mendelian syndromes with
367 neurological manifestations, only *SLC30A10* deficiency (HMNDYT1) is known to be associated
368 with liver disease[59].
369
370 We detect two key aspects of HMNDYT1 – increased circulating liver enzymes and increased
371 hematocrit – exceeding phenome-wide significance in heterozygous carriers of *SLC30A10*
372 Thre95Ile (rs188273166). Among other hepatic phenotypes that have been reported in HMNDYT1
373 cases, we also detect an association with overall liver disease and anemia, but no evidence of
374 hyperbilirubinemia. The neurological aspect of HMNDYT1, parkinsonism and dystonia, is not
375 detectably enriched among Thr95Ile carriers; however, we have limited power and cannot exclude
376 an enrichment. It is therefore intriguing to consider that carrier status of Thr95Ile may represent a
377 very mild manifestation of HMNDYT1.
378

379 **Comparison of Thr95Ile phenotypes to *SLC30A10* common variant phenotypes**

380 Apart from rare variants in *SLC30A10* causing HMNDYT1, Thr95Ile can also be compared to
381 common SNPs in *SLC30A10* that have been associated with phenotypes by GWAS. We find that
382 the minor allele of Thr95Ile is in almost complete linkage with a common intronic SNP associated
383 with increased blood manganese. Other GWAS SNPs in almost perfect linkage with Thr95Ile
384 associate with decreased MCH, increased RBC distribution width, decreased magnesium/calcium
385 ratio, and increased heel bone mineral density (BMD). Decreased MCH could reflect the anemia
386 experienced by HMNDYT1 patients, caused by the closely linked homeostatic regulation of
387 manganese and iron[17]. Increased BMD may reflect the protective role of manganese in bone

388 maintenance[60,61]. Looking for the subset of these phenotypes available in our scan of Thr95Ile, we
389 do find a nominally significant increase in BMD and a suggestive increase in MCH but no
390 detectable increase in erythrocyte distribution width. By contrast, we find that the a common
391 intronic SNP in *SLC30A10* recently reported to associate with liver MRI cT1, a sign of
392 steatohepatitis and fibrosis[36], is in complete linkage with the major allele of Thr95Ile, suggesting
393 an independent genetic mechanism but also providing independent evidence of the role of
394 *SLC30A10* SNPs in liver health.
395
396 The linked GWAS SNPs may be interpreted through two mechanistic hypotheses: first, the
397 associations may all be causally driven by Thr95Ile carriers in the studies, which the GWAS SNPs
398 tag; alternatively, the associations may be driven by effects of the common SNPs themselves,
399 which are noncoding but may influence *SLC30A10* (or another gene in cis) by modulating
400 expression or post-transcriptional regulation; or some combination of both. To distinguish between
401 these, measurements of Mn would need to be available to perform conditional analyses. If the
402 GWAS SNPs have an effect independent of Thr95Ile, *SLC30A10* seems likely (although not
403 certain) to nevertheless be the causal gene at the locus, due to the similarity in phenotypes to
404 HMNDYT1 and Thr95Ile. A putative regulatory mechanism could be through transcriptional or
405 post-transcriptional regulatory elements, as the haplotype includes a SNP (rs2275707) overlapping
406 both the 3'-UTR of *SLC30A10* and H3K4me1 histone modifications characteristic of enhancers
407 active only in brain and liver[62].
408

409 ## Clinical relevance: manganese homeostasis in health and disease

410 Manganese (Mn) is a trace element required in the diet for normal development and function,
411 serving as a cofactor and regulator for many enzymes. However, it can be toxic in high doses;
412 because Mn(II) and Mn(III) can mimic other cations such as iron, it can interfere with systemic
413 iron homeostasis and disrupt in other biochemical processes[63,64]; at the cellular level, it is cytotoxic
414 and poisons the mitochondria by interfering with the electron transport chain enzymes that rely
415 on Fe-S clusters[65]. The hallmark of occupational exposure through inhalation is neurotoxicity
416 manifesting as parkinsonism and dystonia (manganism, or Mn intoxication)[63,64]. Neurotoxicity is
417 an aspect of the Mendelian syndromes caused by loss of function of all three of the hepatic
418 manganese transporters; interestingly, GWAS has also identified a common missense variant in
419 *SLC39A8* as a risk factor for schizophrenia and many other diseases[66,67]; altered function of
420 glycosyltransferases due to manganese overload in the neurons is a proposed mechanism for
421 neurological manifestations of this variant[68]. Because manganese is excreted through the liver into
422 the bile, increased circulating manganese secondary to liver damage may be a contributing factor
423 to the neurological manifestations of chronic hepatic encephalopathy (CHE), known as chronic
424 acquired hepatocerebral degeneration (CAHD)[69-71]. However, liver toxicity is not a hallmark of
425 environmental or occupational exposure. Importantly, of the Mendelian syndromes of genes
426 encoding manganese transporters, only *SLC30A10* (causing HMNDYT1) involves hepatic
427 symptoms[59,72]. Hepatotoxicity in HMNDYT1 is thought to be due to cytotoxic manganese
428 overload within hepatocytes; polycythemia is thought to be caused by upregulation of
429 erythropoietin by manganese; and iron anemia through systemic dysregulation of iron homeostasis
430 by excess manganese[72,73]. Our results indicate that polymorphism in *SLC30A10* is a risk factor for

431 manganese-induced hepatocellular damage, polycythemia, and iron anemia in a much broader
432 population beyond the rare recessive syndrome HMNDYT1.
433
434 The association of *SLC30A10* Thr95Ile with extrahepatic bile duct cancer was unexpected, as it
435 has not been described in conjunction with HMNDYT1. Bile duct cancer (cholangiocarcinoma) is
436 a rare disease (age-adjusted incidence of 1 – 3 per 100,000 per year); cirrhosis, viral hepatitis,
437 primary sclerosing cholangitis, and parasitic fluke infection have been identified as risk factors[74,75].
438 It is unclear whether low levels of manganese in the bile, or high levels of manganese in the
439 hepatocytes and bile duct epithelial cholangiocytes, could be directly carcinogenic; manganese-
440 dependent superoxide dismutase (MnSOD, or SOD2) is a tumor suppressor[76]. A simpler possibility
441 is that cytotoxic manganese overload in hepatocytes and the bile duct epithelium causes localized
442 inflammation that predisposes to cancer through similar mechanisms as other hepatobiliary risk
443 factors. To our knowledge, *SLC30A10* Thr95Ile would be the strongest genetic
444 cholangiocarcinoma risk factor identified to date, being carried by 4% of the extrahepatic bile duct
445 cancer cases in our sample. Because both *SLC30A10* Thr95Ile and extrahepatic bile duct cancer
446 are exceedingly rare, validation of this association in either another very large biobank or in a
447 cohort of cholangiocarcinoma patients will be necessary.
448

449 ## Clinical relevance: genome interpretation

450 Currently, *SLC30A10* Thr95Ile (rs188273166) is listed as a variant of uncertain significance in the
451 ClinVar database[77]. Individuals carrying *SLC30A10* Thr95Ile may benefit from increased
452 surveillance of liver function and blood manganese levels and subsequent treatment if these
453 conditions arise, because evidence from HMNDYT1 patients has demonstrated that chelation

454 therapy combined with iron supplementation is effective at reversing the symptoms of SLC30A10
455 insufficiency[78]. Further studies will be needed to define whether other damaging missense variants
456 or protein-truncating variants in *SLC30A10*, including the variants known to cause HMNDYT1,
457 also predispose to liver disease in their heterozygous state. Because we only observe one
458 homozygous carrier of *SLC30A10* Thr95Ile in our data (who has no diagnosed liver or neurological
459 disease, and unfortunately does not have ALT or AST measurements), further study will also be
460 needed to understand the inheritance model of this association; we cannot determine whether risk
461 in homozygotes is stronger than risk in heterozygotes, unlike cases of HMNDYT1 where identified
462 cases have all been homozygous loss-of-function.
463
464 More broadly, the case of *SLC30A10* fits a pattern of discoveries of recent discoveries showing
465 that recessive Mendelian disease symptoms can manifest in heterozygous carriers of deleterious
466 variants, blurring the distinction between recessive and dominant disease genes and bridging the
467 gap between common and rare disease genetics[79,80]. These discoveries are possible only by
468 combining massive, biobank-scale genotype and phenotype datasets such as the UK Biobank.

469 # Methods

470 ## Sub-population definition and PC calculation

471 Sub-populations for analysis were obtained through a combination of self-reported ethnicity and
472 genetic principal components. First, the White British population was defined using the
473 categorization performed previously by the UK Biobank[81] (Field 22006 value "Caucasian");
474 briefly, this analysis selected the individuals who identify as White British (Field 21000),
475 performed a series of subject-level QC steps (to remove subjects with high heterozygosity or
476 missing rate over 5%, removing subjects with genetic and self-reported sex discrepancies and

477 putative sex chromosome aneuploidies, and removing subjects with second or first degree relatives
478 and an excess of third degree relatives), performed Bayesian outlier detection using the R package
479 aberrant [82] to remove ancestry outliers using principal components (PCs) 1+2, 3+4, and 5+6
480 (calculated from the global UK Biobank PCs stored in Field 22009), selected a subset of SNPs in
481 preparation for PCA by limiting to directly-genotyped (info = 1), missingness across individuals
482 < 2%, MAF > 1%, regions of known long range LD, and pruning to independent markers with
483 pairwise LD < 0.1. Based on this procedure used by the UK Biobank to define the "White British"
484 subset, we defined three additional populations, using other self-reported ancestry groups as
485 starting points (Field 21000 values "Asian or Asian British", "Black or Black British", and
486 "Chinese"). Principal components were estimated in PLINK using the unrelated subjects in each
487 subgroup. We then projected all subjects onto the PCs. For calculation of per-variant allele
488 frequency and missingness thresholds, calculation of LD, and for association analyses performed
489 in PLINK, the unrelated sets were used. For association analyses performed in SAIGE, the related
490 sets were used.
491
492 For validation in an independent population, two other self-reported ethnicity groups with a
493 sufficient number of *SLC30A10* Thr95Ile carriers were assembled, who were not included in
494 "White British" (Field 21000 values "White" subgroup "Irish", and "White" subgroup "Any other
495 white background" or no reported subgroup).
496

497 **Array genotype data for association analysis**

498 Data were obtained from the UK Biobank through application 26041. Genotypes were obtained
499 through array typing and imputation as described previously[81]. For genome-wide association

500 analysis, variants were filtered so that imputation quality score (INFO) was greater than 0.8.
501 Genotype missingness, Hardy-Weinberg equilibrium (HWE), and minor allele frequency (MAF)
502 were then each calculated across the unrelated subset of individuals in each of the four sub-
503 populations. For each sub-population a set of variants for GWAS was then defined by filtering
504 missingness across individuals less than 2%, HWE p-value > $10^{-12}$, and MAF > 0.1%.
505

506 ## Phenotype data

507 For genome-wide analysis, blood biochemistry values were obtained for ALT (Field 30620) and
508 AST (Field 30650) and $\log_{10}$ transformed, consistent with previous genetic studies[3,83], resulting in
509 an approximately normal distribution.
510

511 For phenome-wide analysis, ICD10 codes were obtained from inpatient hospital diagnoses (Field
512 41270), causes of death (Field 40001 and 40002), the cancer registry (Field 40006), and GP clinical
513 event records (Field 42040). A selection of 135 quantitative traits was obtained from other fields,
514 encompassing anthropomorphic measurements, blood and urine biochemistry, smoking, exercise,
515 sleep behavior, and liver MRI; all were inverse rank normalized using the RNOmni R package[84].
516

517 ## Genome-wide association studies of ALT and AST

518 Because of the high level of relatedness in the UK Biobank participants[85], to maximize power by
519 retaining related individuals we used SAIGE software package[86] to perform generalized mixed
520 model analysis for GWAS. A genetic relatedness matrix (GRM) was calculated for each sub-
521 population with a set of 100,000 LD-pruned variants selected from across the allele frequency
522 spectrum. SAIGE was run on the filtered imputed variant set in each sub-population using the

523 following covariates: age at recruitment, sex, BMI, and the first 12 principal components of genetic
524 ancestry (learned within each sub-population as described above). Manhattan plots and Q-Q plots
525 were created using the qqman R package[87]. The association results for each enzyme were meta-
526 analyzed across the four populations using the METAL software package[88] using the default
527 approach (using p-value and direction of effect weighted according to sample size) To report p-
528 value results, the default approach was used. To report effect sizes and standard errors, because
529 the authors of the SAIGE method advise that parameter estimation may be poor especially for rare
530 variants[89], the PLINK software package v1.90[90] was run on lead variants on the unrelated subsets
531 of each subpopulation, and then the classical approach (using effect size estimates and standard
532 errors) was used in METAL to meta-analyze the resulting betas and standard errors. All PLINK
533 and SAIGE association tests were performed using the REVEAL/SciDB translational analytics
534 platform from Paradigm4.
535

536 **Identifying independent, colocalized association signals between the two GWAS**

537 Meta-analysis results for each enzyme were LD clumped using the PLINK software package,
538 v1.90[90] with an $r^2$ threshold of 0.2 and a distance limit of 10 megabases, to group the results into
539 approximately independent signals. LD calculations were made using genotypes of the White
540 British sub-population because of their predominance in the overall sample. Lead SNPs (the SNPs
541 with the most significant p-values) from these "$r^2 > 0.2$ LD blocks" were then searched for proxies
542 using an $r^2$ threshold of 0.8 and a distance limit of 250 kilobases, resulting in "$r^2 > 0.8$ LD blocks"
543 defining potentially causal SNPs at each locus. The "$r^2 > 0.8$ LD blocks" for the ALT results were
544 then compared to the "$r^2 > 0.8$ LD blocks" for the AST results, and any cases where these blocks
545 shared at least one SNP between the two GWAS were treated as potentially colocalized association

546 signals between the two GWAS. In these cases, a representative index SNP was chosen to represent
547 the results of both GWAS by choosing the index SNP of the GWAS with the more significant p-
548 value. Next, these putative colocalized association signals were then distance pruned by iteratively
549 removing neighboring index SNPs within 500 kilobases of each index SNP with less significant
550 p-values (the minimum p-value between the two GWAS was used for the distance pruning
551 procedure.) The Manhattan plot of METAL results with labeled colocalization signals was created
552 using the CMplot R package[91] .
553

554 **Annotation of associated loci and variants**

555 Index SNPs and their corresponding strongly-linked ($r^2 > 0.8$) variants were annotated using the
556 following resources: distance to closest protein-coding genes as defined by ENSEMBL v98 using
557 the BEDTools package[92], impact on protein-coding genes using the ENSEMBL Variant Effect
558 Predictor software package[93]; eQTLs (only the most significant eQTL per gene-tissue
559 combination) from GTEx v8 (obtained from the GTEx Portal) for liver, kidney cortex, and skeletal
560 muscle[94]; a published meta-analysis of four liver eQTL studies[95]; the eQTLGen meta-analysis of
561 blood eQTL studies[96]; and GWAS results from the NHGRI-EBI GWAS Catalog (r2020-01-27)[97],
562 filtered to associations with $p < 5 \times 10^{-8}$.
563

564 **Association ALT- and AST-associated loci with liver disease**

565 Index SNPs were tested for association with any liver disease. A broad liver disease phenotype
566 was constructed by combining all diagnoses mapping to the ICD10 codes K70-K77 (diseases of
567 liver), I85 (esophageal varices), and C22 (malignant neoplasm of liver and intrahepatic bile ducts)
568 in inpatient hospital diagnoses (Field 41270), causes of death (Field 40001 and 40002), cancer

569 registry (Field 40006), and GP clinical event records (Field 42040). For each index SNP and sub-
570 population, two association tests were performed using SAIGE: in the subset of individuals with
571 GP clinical event records available, and in the subset without GP clinical event records available.
572 These pairs of association tests were then meta-analyzed in METAL using the default approach
573 (using p-values) between the with-GP and without-GP subsets. Then, association results were
574 meta-analyzed across the four sub-populations using the same method to obtain the final p-value.
575 To obtain effect sizes and standard errors, the same procedure was performed but using PLINK
576 (on the unrelated subset of each population) and using the classical method in METAL (using
577 effects and standard errors.)
578

579 **Sequencing-based validation of rs188273166 array genotyping**

580 Whole exome sequencing was available for 301,473 of the 487,327 array-genotyped samples.
581 DNA was extracted from whole blood and was prepared and sequenced by the Regeneron Genetics
582 Center (RGC). A complete protocol has been described elsewhere[98]. Briefly, the xGen exome
583 capture was used and reads were sequenced using the Illumina NovaSeq 6000 platform. Reads
584 were aligned to the GRCh38 reference genome using BWA-mem[99]. Duplicate reads were
585 identified and excluded using the Picard MarkDuplicates tool (Broad Institute)[100]. Variant calling
586 of SNVs and indels was done using the WeCall variant caller (Genomics Plc.)[101] to produce a
587 GVCF for each subject. GVCFs were combined to using the GLnexus joint calling tool[102]. Post-
588 variant calling filtering was applied as described previously[98].
589

590 ## Replication of *SLC30A10* Thr95Ile associations

591 ALT and AST association tests were repeated as described for the genome-wide scans, using
592 SAIGE and PLINK, in the "Other White" and "White Irish" populations, for the *SLC30A10*
593 Thr95Ile (rs188273166) variant. Results were meta-analyzed across the two populations. A forest
594 plot was created using the forestplot package in R[103].

595

596 ## Testing linkage of *SLC30A10* Thr95Ile to common GWAS SNPs

597 To test linkage of *SLC30A10* Thr95Ile (rs188273166) to common GWAS SNPs, the GWAS
598 Catalog was searched for all results where "Mapped Gene" was assigned to *SLC30A10*; because
599 of the very relevant phenotype, blood Mn-associated SNP rs1776029, an association that is not in
600 the GWAS Catalog, was also included in the analysis, as well at cT1-associated SNP rs759359281.
601 LD calculations were performed in PLINK, using the White British unrelated subpopulation,
602 between rs188273166 and the GWAS SNPs with the options --r2 dprime-signed in-phase with-
603 freqs --ld-window 1000000 --ld-window-r2 0. For rs1776029, an additional Fisher's exact test was
604 performed to determine the confidence interval of the enrichment of rs188273166 on the
605 rs1776029 haplotype. The linked alleles from PLINK were then used in conjunction with the effect
606 allele from the reported papers to determine the direction of effect. The GWAS Atlas website[32]
607 was used (the PheWAS tool) to determine the direction of effect for the linked alleles from the
608 original paper; in cases where the original paper from the GWAS Catalog did not report a direction
609 of effect, other papers for the same phenotype and SNP from GWAS Atlas were used to determine
610 the direction of effect and cited accordingly. Reference epigenome information for the GWAS
611 SNPs was obtained by searching for rs1776029 in HaploReg v4.1[104].

612

613 ## Phenome-wide association study of *SLC30A10* Thr95Ile

614 A phenome-wide association study of *SLC30A10* Thr95Ile (rs188273166) was performed by
615 running SAIGE and PLINK against a set of ICD10 diagnoses and quantitative traits, obtained as
616 described above, and meta-analyzed using METAL as described above for the test of association
617 with liver disease. ICD10 diagnoses were filtered to include only those at a three-character
618 (category), four-character (category plus one additional numeral), or "block" level that were
619 frequent enough to test in both subpopulations and without significant collinearity with the sex
620 covariate: at least 100 diagnoses overall, at least 10 diagnoses in both the with- and without-GP
621 data subgroups, and at least 10 diagnoses in both men and women. This resulted in 4,533 ICD10
622 codes to test, serving as the multiple hypothesis burden. For 5 of these ICD10 code phenotypes,
623 the first step of SAIGE (fitting the null logistic mixed model to estimate the variance component
624 and other model parameters) failed before the association test; for these phenotypes, only PLINK
625 was run.

626

627 ## Bioinformatic analysis of *SLC30A10* Thr95Ile

628 To visualize Thr95Ile on the protein sequence of SLC30A10, UNIPROT entry Q6XR72
629 (ZN10_HUMAN) was accessed[53]. In UNIPROT, natural variants causing HMNDYT1[22,24,38] and
630 mutagenesis results[38,54,105] were collated from the literature and highlighted. CADD score v1.5[106]
631 was downloaded from the authors' website. SIFT score was obtained from the authors' website
632 using the "dbSNP rsIDs (SIFT4G predictions)" tool[107]. PolyPhen score and multiple species
633 alignment was obtained from the authors' website using the PolyPhen-2 tool[108].

634

635 **Immunofluorescence of SLC30A10 localization in cultured cells**

636 HeLa cells (ATCC®, Manassas, VA) were grown in Eagle's Minimum Essential Medium
637 (ATCC®, Manassas, VA) containing 10% fetal bovine serum (Gibco, Carlsbad, CA) at 37°C and
638 5% $CO_2$. All plasmid transfections were performed using Lipofectamine™ 2000 (Invitrogen,
639 Carlsbad, CA) and Opti-MEM (Gibco, Grand Island, NY) according to manufacturer's
640 specifications. FLAG-tagged SLC30A10 plasmid constructs designed with a linker sequence in
641 pCMV6-AN-3DDK (Blue Heron Biotech, Bothell, WA) included wild type, del105-107, L89P,
642 T95I, and used an empty vector for one of the negative controls.

643

644 HeLa cells were grown on 8-chambered slides for 48 hours post-transfection. IF procedures were
645 performed at room temperature unless otherwise noted. HeLa cells were rinsed in PBS, fixed with
646 4% paraformaldehyde (in water) (Electron Microscopy Sciences, Hatfield, PA) for 10 min, rinsed
647 in 4°C PBS, and permeabilized for 5 minutes with 0.1% Triton X-100 (Sigma-Aldrich, St. Louis,
648 MO). After rinsing in PBS and blocking in 2% BSA (in PBS) (Jackson ImmunoResearch, West
649 Grove, PA) for 30 minutes, the cells were stained with 2% BSA blocking solution containing
650 monoclonal ANTI-FLAG® M2-FITC, Clone M2 (dilution 1:100; Sigma-Aldrich, St. Louis, MO)
651 and Calnexin Monoclonal Antibody, Clone AF18 (dilution 1:100; Invitrogen, Carlsbad, CA). After
652 three final washes in PBS, mounting medium with DAPI (Vector Laboratories, Burlingame, CA)
653 was added and sealed under a coverslip with nail polish. Images were captured with the REVOLVE
654 Echo microscope at 20X magnification.

655

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660 ## Author Contributions

661 A.D., A.F.C., L.W., M.P., and P.N. performed computational analyses; C.Q. and H.C.T. performed
662 experiments; L.W. wrote the manuscript; all authors interpreted results and edited the manuscript.

663 ## Competing Interests

664 The authors are all employees of Alnylam Pharmaceuticals, Inc.

665

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667

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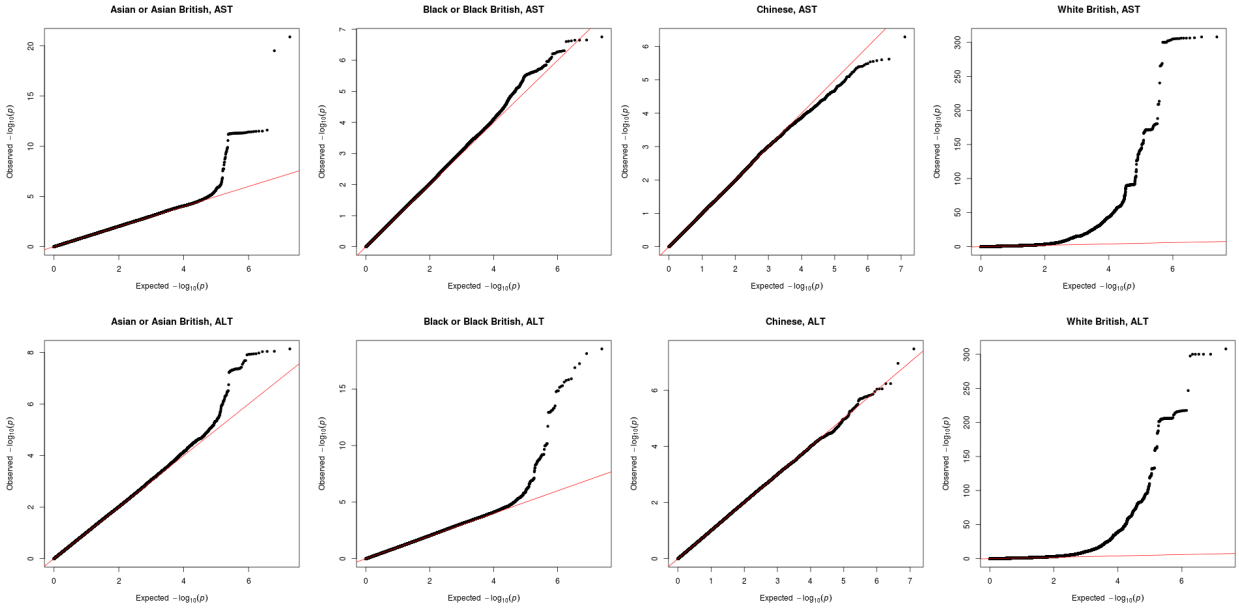
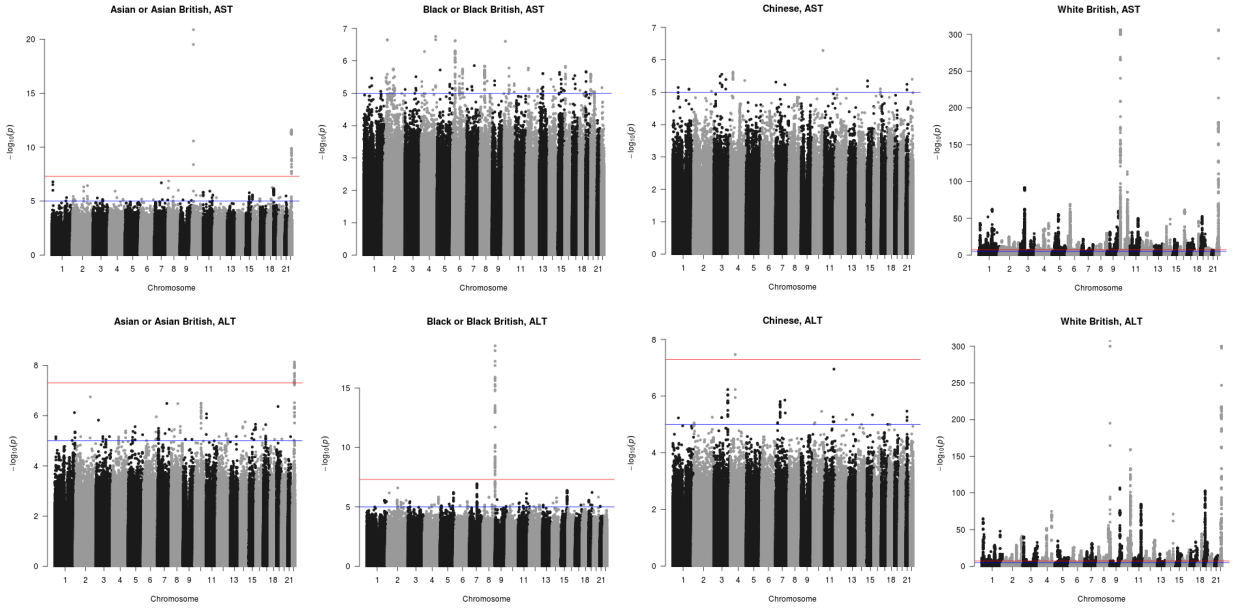
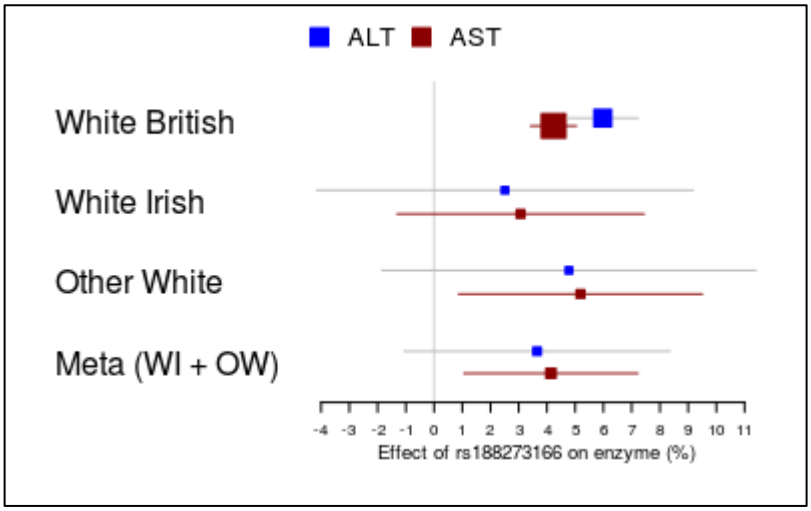
# Supplementary Figures



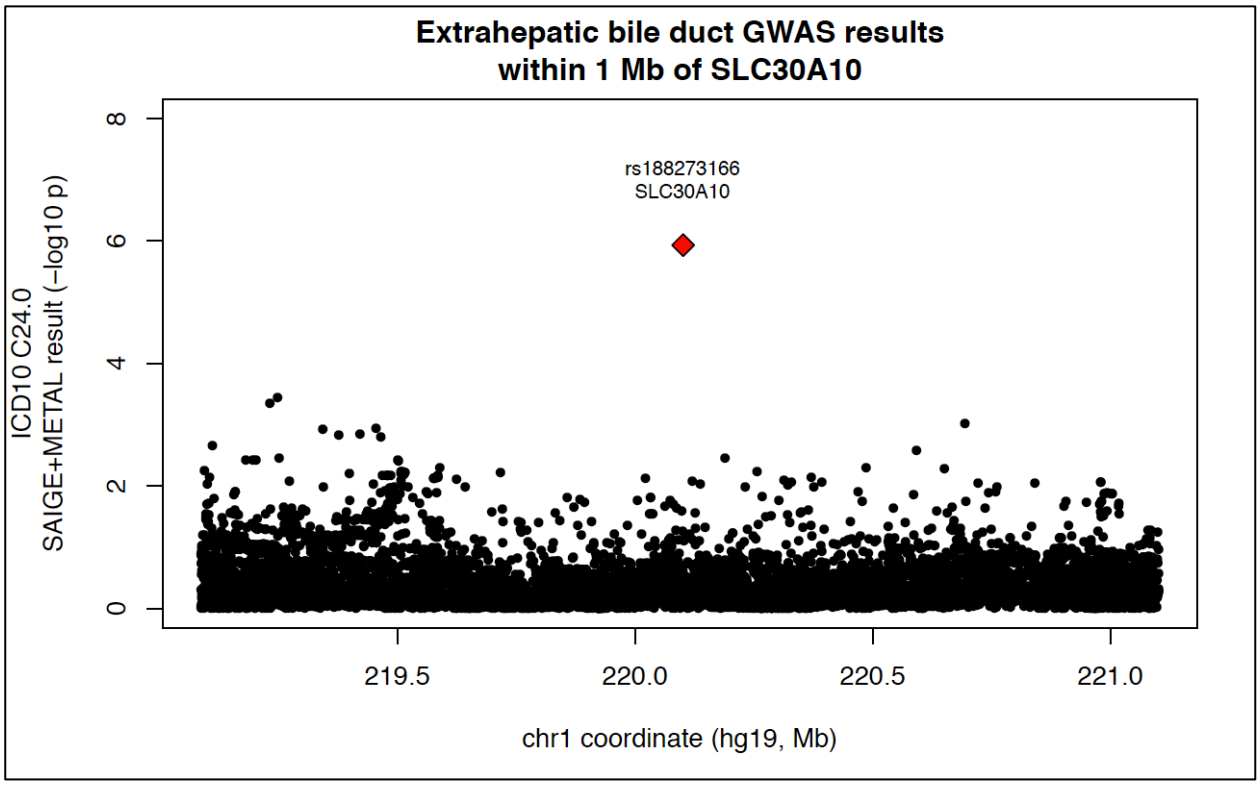

**Supplementary Figure 1:** Q-Q plots of GWAS p values (from SAIGE) for each sub-population and enzyme.




**Supplementary Figure 2:** Manhattan plots of GWAS p values (from SAIGE) for each sub-population and enzyme.




**Supplementary Figure 3:** Forest plot of *SLC30A10* Thr95Ile (rs188273166) association with ALT and AST in White British, White Irish, and Other White populations, and meta-analysis results (in METAL) of White Irish and Other White association results, combined. Boxes show effect size estimates from PLINK, and error bars show 95% confidence intervals.




**Supplementary Figure 4:** Association results (p value from SAIGE followed by METAL) for ICD10 diagnosis C24.0, extrahepatic bile duct cancer, in the *SLC30A10* gene and 1 megabase flanking sequence.