A rare genetic variant in the manganese transporter *SLC30A10* and elevated liver enzymes in the general population

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Abbreviations:

CCHS: Copenhagen City Heart Study

CGPS: Copenhagen General Population Study

PDFF: Proton density fat fraction

PNPLA3: Patatin-like phospholipase domain-containing protein 3

SLC30A10: Solute Carrier Family 30 Member 10

UKB: UK Biobank

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Abstract

Background: A genetic variant in the manganese transporter *SLC30A10* (rs188273166, p.Thr95Ile) was associated with increased plasma alanine transaminase (ALT) in a recent genome-wide association study in the UK Biobank (UKB). The aims of the present study were to test the association of rs188273166 with ALT in an independent cohort, and to begin to assess the clinical, hepatic, and biochemical phenotypes associated with the variant.

Methods: We included n=334,886 white participants from UKB, including 14,462 with hepatic magnetic resonance imaging (MRI), and n=113,612 individuals from the Copenhagen City Heart Study and the Copenhagen General Population Study combined.

Results: Genotyping *SLC30A10* p.Thr95Ile identified 816 heterozygotes in the UKB and 111 heterozygotes in the Copenhagen cohort. Compared to noncarriers, heterozygotes had 4 U/L and 5 U/L higher levels of ALT in the UKB and Copenhagen cohort, respectively, and 3 U/L higher plasma aspartate transaminase and gamma glutamyl-transferase in the UKB. Heterozygotes also had higher corrected T1 on liver MRI, a marker of hepatic inflammation ($P=4\times10^{-7}$), but no change in MRI-quantified steatosis (P=0.57). Plasma manganese was within the normal range in nine heterozygotes that provided new blood samples. *SLC30A10* p.Thr95Ile heterozygotes had an eight-fold increased risk of biliary tract cancer in UKB ($P=5\times10^{-7}$), but this association was not replicated in the Copenhagen cohort.

Conclusions: *SLC30A10* p.Thr95Ile was associated with elevated liver enzymes in two large general population cohorts, and with MRI-quantified hepatic inflammation.

Introduction

Chronic liver disease is a major cause of morbidity and mortality worldwide. The main drivers of the disorder are obesity-associated hepatic steatosis and inflammation, chronic excessive alcohol consumption, and viral hepatitis. Heritable factors account for approximately half of the interindividual variation in risk of chronic liver disease(1, 2). So far, exome- and genome-wide association studies (GWAS) have identified 15 common sequence variations that affect the risk of the disorder(3-10).

A recent GWAS identified variants at over 200 different genetic loci to be associated with plasma levels of alanine transaminase (ALT) and aspartate transaminase (AST), biochemical markers of liver cell injury(11). The variant with the largest absolute ALT and AST-increasing effect was a rare missense variant in the gene encoding Solute Carrier Family 30 Member 10 *(SLC30A10)*. The variant causes a change from threonine to isoleucine at amino acid residue 95 of the encoded protein (p.Thr95Ile). The ALT-increasing effect of the variant was approximately twice that of *PNPLA3* p.Ile148Met, a well-known genetic risk factor for fatty liver disease that confers a three to four-fold higher risk of chronic liver disease. The effect of *SLC30A10* p.Thr95Ile on liver disease is unknown.

The aims of the present study were first to validate the association of *SLC30A10* p.Thr95Ile with plasma levels of liver enzymes in an independent cohort, and second, to test whether the variant confers an increased risk of chronic liver disease by studying its effect on the clinical, hepatic, and biochemical phenotypes associated with chronic liver disease.

Methods

Cohorts

For this study, we included 334,886 participants from the UKB, and 113,612 participants from the Copenhagen General Population Study (CGPS) and the Copenhagen City Heart Study (CCHS) combined, referred to here as the Copenhagen cohort.

UKB

The UKB is a prospective cohort study consisting of approximately half a million individuals between 40 and 69 years of age from across the United Kingdom(12). Participants were identified through the National Health Services central registers, and baseline assessment was between 2006 and 2010. Participants were evaluated through a questionnaire, an interview and a physical examination, and blood samples were collected for analyses. Genetic data were available for approximately 490,000 participants from the UKB. The quality control pipeline for genotype data has been described in detail previously(13). In short, genetic data underwent marker-based and sample-based control including checks for population substructure based on principal component analyses. Furthermore, the pipeline included control for missingness, heterozygosity, sex mismatch and relatedness. We included 334,886 non-related individuals of self-reported white, British descent.

Copenhagen cohort

The CGPS and the CCHS are both prospective cohort studies of the Danish general population, initiated in 2003 and 1976-1978, respectively(14, 15). Participants aged between 20 and 100+ years were randomly selected based on the national Danish Civil Registration System and invited to participate in the studies. All participants were white and of Danish descent. Baseline data were obtained from a questionnaire, a physical examination and from blood samples, including DNA

extraction. We included 103,321 individuals from the CGPS and 10,291 individuals from the CCHS.

Genotyping

SLC30A10 rs188273166 and *PNPLA3* rs738409 were genotyped by GWAS chip (UKB Axiom Array and UK BiLEVE Axiom Array, Affymetrix) in UKB, and by TaqMan assays (ABI PRISM 7900HT Sequence Detection System, Applied Biosystems) in the Copenhagen cohorts. Genotype call clustering in the UK Biobank was assessed using Scattershot at http://mccarthy.well.ox.ac.uk/static/software/scattershot/. All heterozygotes in the Copenhagen cohort were verified by re-genotyping. Exome data used for principal components analyses in the Copenhagen cohort were derived from Illumina Exome chip.

Biochemistry

Non-fasting plasma levels of alanine transaminase (ALT), aspartate transaminase (AST), gammaglutamyl transferase (GGT) and albumin were measured at the time of study entry using standard hospital assays (Beckman Coulter, High Wycombe, United Kingdom, in UKB; Konelab, Helsinki, Finland, and Boehringer Mannheim, Mannheim, Germany in the CGPS and the CCHS). Plasma AST was only available in a subset of 9,180 participants from the Copenhagen studies. Plasma manganese was measured using inductively coupled mass spectrometry (iCAP™ RQ ICP-MS, Thermo Fisher, Waltham, MA, USA). Manganese was measured in 107 participants from the Copenhagen cohort (50 noncarriers and 57 heterozygote carriers of the *SLC30A10* p.Thr95Ile variant) using thawed plasma samples collected in ethylenediaminetetraacetic acid (EDTA) tubes. However, EDTA and other tubes routinely used in the context of large-scale biobanks can have manganese contamination(16). Therefore, heterozygous carriers of *SLC30A10* p.Thr95Ile in the Copenhagen General Population Study (who were alive and below 80 years of age in December

2020) were invited, by email, to a new blood draw taken in a trace element free tube (Beckton Dickinson, Franklin Lakes, NJ, USA). Of 70 invited heterozygotes, nine provided blood. Manganese was also measured in plasma from 33 anonymized blood samples received for routine analyses in our lab, all drawn in trace-element free tubes. These samples were defined as *SLC30A10* p.Thr95Ile noncarriers. A normal range for plasma manganese was derived from the literature(17).

Hepatic imaging

Characterization of corrected T1 (cT1) in the UK Biobank cohort has previously been published(18-20). Briefly, the MRI sequence is part of the LiverMultiScan© protocol from Perspectum Diagnostics (UK) which forms part of the UKB abdominal imaging protocol. The protocol measured T2*, T1, and proton-density fat fraction (PDFF). The T1 relaxation time reflects extracellular fluid and is characteristic of fibrosis and inflammation. Corrected T1 relaxation time is obtained by correcting T1 for iron content as assessed by T2*. Measurements of hepatic computed tomography (CT) attenuation were available in 7,201 individuals from the CGPS(21, 22).

Other variables

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Alcohol consumption was self-reported current intake of alcohol in units per week (1 unit=12g of alcohol). Diabetes mellitus was defined as individuals with one or more of the following ICD codes: ICD-10 E10, E11, E13, or E14 and/or ICD-8 249 or 250.

Clinical endpoints

In UKB, we extracted International Classification of Diseases, tenth revision (ICD-10) codes from in-hospital records, causes-of-death registries and cancer registries (UKB data fields 41202, 41204, 40001, 40002, 40006, and 40013). The National Health Service initiated the use of ICD-10 codes in April of 1995. In the Copenhagen cohort, ICD-8 and ICD-10 codes were collected from the national

Danish Patient Registry and the national Danish Causes of Death Registry from January 1, 1977, to December 13th, 2018, and from the Danish Cancer Registry from 1943 through December 31, 2016 (last update of the registry). The National Danish Patient Registry has information on all patient contacts with all clinical hospital departments in Denmark, including emergency wards and outpatient clinics (from 1994). The national Danish Causes of Death Registry contains data on the causes of all deaths in Denmark, as reported by hospitals and general practitioners. The Danish cancer registry contains complete information on all cancer events for all Danish citizens, and all cancers in the registry are histologically verified. We defined 'any liver disease' as participants having received at least one of the following ICD-10 codes: K70-K77 (diseases of the liver), C22.0 or C22.9 (hepatocellular carcinoma) and/or (in the Copenhagen cohort) ICD Eighth Revision (ICD-8) 570-573 (diseases of the liver). Cirrhosis was defined as anyone having received K70.3 (alcoholic cirrhosis) and/or K74.6 (unspecified cirrhosis of liver) and/or (in the Copenhagen cohort) as individuals with ICD-8 codes 57109 (alcoholic cirrhosis), 57192 (unspecific cirrhosis), and/or 57199 (cirrhosis of nonalcoholic causes). Biliary tract cancer was defined as anyone having received one of the following ICD-10 codes: C22.1 (intrahepatic bile duct carcinoma), C23 (malignant neoplasm of gallbladder), C24 (malignant neoplasm in other and unspecified parts of biliary tract). Viral hepatitis was defined as anyone having received one of the following ICD-10 codes B18 (chronic viral hepatitis), B17.1 (acute hepatitis C) and/or (in the Copenhagen cohort) ICD-8 57193 (chronic viral hepatitis). Neurological disease was defined by ICD-10 codes G0 to G9 in either cohort.

Statistical analyses

Statistical analyses were performed using R version 3.6.0. Genotypes were coded as 0 for noncarriers and 1 for heterozygote carriers of *SLC30A10* p.Thr95Ile. There was a single homozygous carrier of the variant in the UKB. For statistical analyses, this individual was coded 1,

and thus pooled with the heterozygotes. Kruskal-Wallis and chi^2 tests where used to test if continuous and binary variables were randomly distributed between genotypes. Associations with continuous and categorical variables were assessed using linear or logistical regression adjusted for age and sex, and (in the UKB) additionally adjusted for principal GWAS components 1 through 10 (to account for potential population stratification). As a sensitivity analysis, the effect of viral hepatitis on risk of liver disease and biochemical liver markers (ALT, AST, bilirubin, alkaline phosphatase and albumin) was tested first by including viral hepatitis status in the regression models and then in a separate analysis by excluding all known hepatitis cases before performing regressions. Interaction between genotype and other variables was tested by including a product term in the regression. To test for population stratification in the CCHS and the CGPS, genetic principal component 1 was plotted against principal component 2, stratified by *SLC30A10* genotype or biliary tract cancer status. The genotypes used for the principal components analyses were derived from an Illumina Exome chip that had been previously genotyped in 7,579 and 10,302 participants from the CCHS and CGPS, respectively.

Results

Baseline characteristics for the UKB and the Copenhagen cohort, stratified by *SLC30A10* p.Thr95Ile genotype, are shown in Table 1. Of the 334,886 UKB participants, 816 were heterozygous for p.Thr95Ile, and a single person was homozygous for the variant. Among the 113,612 participants from the Copenhagen cohort, 111 were heterozygous for p.Thr95Ile. Baseline characteristics did not differ by *SLC30A10* genotype in either cohort, except for viral hepatitis status, which was more prevalent among heterozygous carriers in the Copenhagen cohort. Principal components analyses did not reveal any evidence of population stratification in the Copenhagen cohorts. *SLC30A10* p.Thr95Ile heterozygotes clustered with the rest of the participants (Supplemental Figure 1).

Biochemical markers of liver disease

SLC30A10 p.Thr95Ile was associated with plasma ALT in both cohorts (Table 2). In UKB, median ALT was 24 U/L in *SLC30A10* p.Thr95Ile heterozygotes and 20 U/L in noncarriers (absolute difference: 4 U/L, relative difference: 20%). The corresponding values in the Copenhagen cohort were 25 U/L and 20 U/L (absolute difference: 5 U/L, relative difference 25%). The average ALT-increasing effect of *SLC30A10* p.Thr95Ile heterozygosity was larger than that of *PNPLA3* p.Ile148Met, a well-known genetic risk factor for fatty liver disease (Figure 1). For example, homozygous carriers of the steatogenic methionine allele of *PNPLA3* p.Ile148Met had 2 U/L higher median ALT compared to noncarriers in the UKB, corresponding to half of the ALT-increase conferred by *SLC30A10* p.Thr95Ile in the same cohort (Figure 1). Among *SLC30A10* p.Thr95Ile heterozygotes, 14% had plasma ALT above the upper limit of normal (32 U/L for women and 44 U/L for men)(23), compared to 9% among noncarriers. There were no interactions between

SLC30A10 p.Thr95Ile and *PNPLA3* p.Ile148Met on plasma ALT in either cohort (both P-values for interaction >0.2). There was a marginally significant interaction between *SLC30A10* p.Thr95Ile and BMI on plasma ALT in the Copenhagen cohort (P = 0.03), and with alcohol intake on ALT in the UKB (P = 0.02). However, neither interaction was replicated in the other cohort. Heterozygous carriers of p.Thr95Ile had 3 U/L higher AST, 3 U/L higher GGT, and marginally lower albumin in UKB. The variant was not associated with these biomarkers in the Copenhagen cohort. Adjusting for viral hepatitis in the regression models or excluding individuals with a diagnosis of viral hepatitis did not materially alter the associations between *SLC30A10* p.Thr95Ile and biochemical liver markers (Supplemental Table 1 and 2).

Plasma manganese

Manganese was measured in plasma drawn in trace element free tubes from nine heterozygotes (44 to 79 years of age, five women and four men) from the Copenhagen cohort, and in 33 noncarriers (Supplemental Figure 2). Plasma manganese was within the normal range (0.5 µg/L to 1.30 µg/L) for all nine heterozygotes. Median plasma manganese was 0.75 µg/L (interquartile range, 0.64-0.81 µg/L) in heterozygotes, and 0.81 µg/L (0.70-0.92 µg/L) in noncarriers (P-value for difference between heterozygotes and noncarriers=0.25). To increase power, plasma manganese was also measured in thawed plasma from 57 heterozygotes and 50 noncarriers from the Copenhagen cohort. These plasma samples had been drawn and stored in EDTA-tubes. No statistically significant association was seen between *SLC30A10* p.Thr95Ile and plasma manganese levels in this subsample (Supplemental Figure 3).

Red blood cell traits

Polycythemia is a hallmark of hypermanganism caused by mutations in *SLC30A10*(24,25) We therefore tested the associations of *SLC30A10* p.Thr95Ile with red blood cell traits (Table 2). Compared to noncarriers, heterozygotes had slightly elevated median hemoglobin concentrations in both the UKB and Copenhagen Cohorts (by 0.2 and 0.3 g/dL, respectively). Heterozygous carriers also had slightly elevated red blood count and hematocrit in the UKB and higher MCV in the Copenhagen cohort.

Hepatic MRI

Liver MRI cT1 (a marker of hepatic inflammation and fibrosis) was available in 14,462 UKB participants. Compared to noncarriers, *SLC30A10* p.Thr95Ile heterozygotes had higher liver cT1 (Supplemental Figure 4). The median (interquartile range) was 743 ms (726 ms to 763 ms) in heterozygotes and 684 (653 ms to 719 ms) in noncarriers (P-value for difference= 5×10^{-7}). *SLC30A10* p.Thr95Ile was not associated with hepatic steatosis (P=0.57 for association with PDFF).

Hepatic CT-attenuation

Hepatic iron accumulation is known to associate with increased CT attenuation(24). We therefore wondered if *SLC30A10* p.Thr95Ile, which is hypothesised to affect hepatic manganese accumulation, was associated with hepatic CT-attenuation (Supplemental Figure 5). Measurements of CT-attenuation were available in n=7,201 participants from the Copenhagen cohort. Of the 12 *SLC30A10* p.Thr95Ile heterozygotes with available CT-attenuation measurements, nine had hepatic attenuation values above the BMI-adjusted median. One heterozygote had a very high CT-attenuation value of 81.3 Hounsfield Units, corresponding to the 99.5th BMI-adjusted percentile.

Mean Hounsfield Units were 60.7 in noncarriers and 64.2 in p.Thr95Ile heterozygotes (P-value for difference between heterozygotes and noncarriers=0.17).

Risk of liver disease

Given its robust ALT-increasing effect, we tested the association of *SLC30A10* p.Thr95Ile with risk of hepatic endpoints (Table 3). There were 2,438 and 2,074 individuals with a diagnosis of 'any liver disease' in the UKB and Copenhagen studies, respectively. The corresponding numbers for cirrhosis were 342 and 503. *SLC30A10* p.Thr95Ile heterozygotes had an OR for 'any liver disease' of 1.73 (95 % CI: 0.95-3.13) as compared to noncarriers in UKB. In the Copenhagen studies, only a single of the 111 heterozygotes had a diagnosis of 'any liver disease', yielding an OR of 0.49 (95% CI: 0.07-3.51) as compared to noncarriers. The corresponding ORs for cirrhosis were 2.17 (95% CI: 0.54-8.75) in UKB and 2.05 (95% CI: 0.28-14.72) in the Copenhagen studies. There were 163 cases of viral hepatitis in the Copenhagen cohort. Two cases occurred in heterozygous carriers, yielding an OR of 12.8 (95 % CI: 3.1-52.3) and a P-value of 0.0004. There were 167 cases of viral hepatitis in the UKB, all in noncarriers. Risk of liver disease did not change when adjusting analyses for viral hepatitis status nor when excluding hepatitis cases (Supplemental Table 3 and 4).

Risk of biliary tract cancer

There were 297 and 122 individuals with a diagnosis of biliary tract cancer in the UKB and Copenhagen cohort, respectively. Heterozygous carriers of p.Thr95Ile had an OR for biliary tract cancer of 7.80 (95% CI, 3.50-17.40) as compared to noncarriers in UKB. None of the heterozygotes in the Copenhagen cohort developed biliary tract cancer. Risk of biliary cancer did not change when adjusting the analysis for viral hepatitis status nor when excluding hepatitis cases from the analysis.

(Supplemental Table 3 and 4). The biliary tract cancer cases clustered with the rest of the participants in principal components analyses in the Copenhagen cohort, indicating that population stratification did not influence these analyses (Supplemental Figure 1).

Neurological disease

Hypermanganism due to homozygosity or compound heterozygosity for mutations in *SLC30A10* cause various neurological symptoms, including dystonia and parkinsonism(25, 26). We therefore wondered if *SLC30A10* p.Thr95Ile confers an increased risk of neurological disease (Supplemental Table 1). In the UKB, heterozygotes for the variant had ORs for 'episodic and paroxysmal disorders' and for 'other disorders of the nervous system' of 1.66 (95% CI, 1.17-2.37) and 2.02 (95% CI, 1.08-3.78), respectively, as compared to noncarriers. These associations were not replicated in the Copenhagen cohort (Supplemental Table 5).

Discussion

We characterized a rare missense variant in the manganese transporter *SLC30A10* recently found to associate with elevated plasma ALT in the UKB. We first validated its association with ALT in an independent cohort of 113,612 individuals. The concentration of plasma ALT was 4-5 U/L higher in heterozygous carriers of *SLC30A10* p.Thr95Ile as compared to noncarriers, an effect size that is substantially larger than those seen for other genetic risk factors for chronic liver disease, including the well-known steatogenic variant *PNPLA3* p.Ile148Met. Using two cohorts totaling 448,498 white individuals, we showed that *SLC30A10* p.Thr95Ile heterozygotes had higher MRI-quantified hepatic inflammation, hemoglobin concentrations, red blood count, and hematocrit and higher risk of biliary tract cancer and neurological disease as compared to noncarriers.

The association of *SLC30A10* p.Thr95Ile with liver enzyme levels was first reported in 2021 in a GWAS of biochemical traits in the UKB(11). Another very recent UKB GWAS identified the same association with elevated plasma ALT and replicated it in an independent cohort of 133,000 individuals(27). In an earlier GWAS, Parisinos and colleagues found a common intronic variant in *SLC30A10* (rs759359281) to be associated with increased hepatic inflammation in 14,440 UKB participants with hepatic cT1 measurements. The variant was also associated with increased ALT and AST levels(4). This association pattern (elevated ALT and AST, and higher cT1) mirrors that seen for *SLC30A10* p.Thr95Ile. The ALT-increasing isoleucine allele of *SLC30A10* p.Thr95Ile is in linkage with the liver cT1-decreasing major allele of rs759359281 (D'=1), suggesting that there are two or more independent variants with effects on hepatic inflammation at the *SLC30A10* locus. These previous findings align with those from the present study. In other words, the association between genetic variation in *SLC30A10* and increased liver damage as measured by biochemical and imaging markers of liver disease is robust and reproducible.

The role of *SLC30A10* in manganese metabolism was discovered in 2012, when two groups simultaneously reported that homozygosity or compound heterozygosity for mutations in the gene caused hypermanganism with dystonia-1, a disorder characterized by the accumulation of manganese in the liver and brain, polycythemia, early-onset cirrhosis, parkinsonism-like dystonia, increased ALT levels and elevated manganese concentration in the blood(25, 26). The disorder has since been described in additional case reports(28) and in reviews(29). *SLC30A10* is expressed at the highest levels in the liver, followed by intestine and brain, and encodes a 485 amino acid transmembrane manganese transporter. In hepatocytes, SLC30A10 effluxes manganese across the hepatocanalicular membrane into the bile(30). Mice deficient for *slc30a10* have markedly elevated manganese levels in their blood and tissues, most notably in the liver, brain, bone, and duodenum(30, 31). High concentrations of manganese are cytotoxic owing to multiple effects, including competition with other cations for binding to key intracellular proteins, disruption of DNA replication and transcription, and interference with mitochondrial function(29).

We hypothesize that the phenotype associated with *SLC30A10* p.Thr95Ile heterozygosity represents an attenuated form of *SLC30A10* deficiency, leading to mild accumulation of manganese This aligns with the attenuated phenotypes observed in heterozygote carriers of mutations in the genes underlying hemochromatosis(32) and Wilson's disease(33), two other recessive disorders characterized by metal accumulation in tissues. Parents of patients with *SLC30A10* deficiency (who are obligate heterozygotes) have mildly elevated plasma manganese levels as compared to noncarriers, supporting that heterozygosity for *SLC30A10*-mutations associates with an attenuated phenotype(26). The observation that *SLC30A10* p.Thr95Ile associates with mildly elevated hemoglobin, mirroring the polycythemia seen in patients with *SLC30A10* deficiency, further supports the notion that p.Thr95Ile associates with an attenuated form of *SLC30A10* deficiency.

The clinical impact of *SLC30A10* p.Thr95Ile heterozygosity is unclear. Given that very few of the heterozygous carriers of the variant developed liver disease in our study, the clinical value of screening or monitoring these individuals for liver disease appears to be limited. We speculate that the variant represents a moderate risk factor, similar to other more common genetic risk variants like *PNPLA3* p.Ile148Met and *TM6SF2* p.Glu167Lys. Based on its effect on plasma ALT, the risk increasing effect of *SLC30A10* p.Thr95Ile heterozygosity is likely comparable to that seen in homozygous carriers of the *PNPLA3* and *TM6SF2* variants as compared to noncarriers (i.e. approximately 3-4 fold higher risk). It is possible that other rare loss-of-function variants in *SLC30A10* exist and that these are associated with even greater risk of liver damage than the p.Thr95Ile variant. Querying the Gnomad-database reveals a single other missense variant that is more common than p.Thr95Ile (p.His431Gln with a frequency of 0.003 in African Americans), and eight different nonsense variants, all with lower frequency than 1×10^{-5}.

Establishing an association of *SLC30A10* p.Thr95Ile with a manganese phenotype would support that the variant impacts manganese transport *in vivo*. To test this, we measured plasma manganese in nine heterozygous carriers that were invited for a fresh blood draw taken in trace element free tubes. The concentration of plasma manganese in these heterozygotes was within the normal range and did not differ from concentrations in noncarriers. To increase power, we also measured manganese levels in thawed plasma from 57 heterozygotes and 50 noncarriers that had been drawn in standard EDTA-tubes. No association between *SLC30A10* genotype and manganese concentration was observed in these samples. Taken together, we did not find an association between the *SLC30A10* p.Thr95Ile variant and plasma levels of manganese. There are at least two potential explanations for this. First, it is possible that the effect size is too small to detect without a larger sample size. Second, the variant's primary effect may be on intracellular accumulation of manganese in hepatocytes and/or neurons, without affecting plasma levels of the metal(34).

Measuring manganese in blood or plasma requires that the sample is drawn in trace-element free tubes, because other, routinely used tubes may have substantial manganese contamination(16). Future studies attempting to test the association of *SLC30A10* p.Thr95Ile with plasma manganese levels should therefore ideally be done on fresh samples drawn in trace element free tubes, necessitating recall-by-genotype of participants. Indirectly supporting an association of *SLC30A10* p.Thr95Ile with plasma manganese is the observation that a nearby common variant associates strongly with plasma manganese levels(35). As noted by Ward and colleagues(27), the ALT-increasing isoleucine-allele of p.Thr95Ile is linked with the manganese-increasing allele of the common variant ($r2 = 0.005$, $D' = 0.98$).

The phenotype associated with homozygosity for *SLC30A10* p.Thr95Ile remains unclear. Based on the minor allele frequency of the variant, the expected prevalence of homozygosity is about one per million. In the UKB, the only homozygous carrier of the variant is a 60-year-old man with a BMI of 32 and no diagnoses of liver or neurological disease. Unfortunately, plasma ALT or other biochemical analyses are not available for this individual. The fact that he was seemingly healthy at 60 years of age argues against p.Thr95Ile homozygosity causing severe, early-onset hypermanganism. However, late onset of symptoms has been reported in some patients with *SLC30A10* deficiency. For example, a patient in one of the initial reports of the disorder presented with neurological symptoms at age 57(25).

There are limitations to our study that should be considered. Despite the large sample sizes of the included cohorts, the number of clinical cases was moderate, limiting statistical power for these endpoints. Another limitation is that endpoints based on ICD-codes inevitably suffer from some degree of misclassification. That said, the validity of the ICD-based liver disease endpoints is supported by the fact that well-known genetic risk factors for chronic liver disease, including *PNPLA3* p.Ile148Met, associate with these endpoints in both cohorts, with effect sizes that are

comparable to those seen in other studies that use imaging or histology to define endpoints(15). Misclassification was likely negligible for the biliary tract cancer cases in the Copenhagen cohort because these were extracted from the Danish Cancer Registry, a registry that includes only histologically verified cancers. Finally, our study included participants of white European ancestry from the Danish and British general populations, potentially limiting its generalizability to other ethnicities or countries.

Some differences between the UKB and Copenhagen cohort phenotypes and genotypes should also be noted. First, the prevalence of liver disease was slightly higher in the Copenhagen cohort than in the UKB (2% versus 0.7%). A potential explanation to this is that the response rate in the Copenhagen cohort is higher than in the UKB (50-70% vs ~6%), indicating that healthy user bias is likely more pronounced in the UKB (i.e. individuals with severe liver disease are less likely to show up to the baseline examination in the UKB). Furthermore, the follow-up time is longer in the Copenhagen cohort, which might lead to a higher number of participants developing liver disease. The frequency of *SLC30A10* p.Thr95Ile also differed somewhat between the two cohorts (0.05% in the Copenhagen cohort versus 0.1% in the UKB). This is likely attributable to the rareness of the variant, and chance. Ultra-rare variants can differ substantially in frequency between neighboring geographic regions due to founder effects or genetic drift. Finally, we observed that *SLC30A10* p.Thr95Ile was associated with higher risk of viral hepatitis in the Copenhagen cohort. This association was, however, based on only two cases and was not replicated in the UKB, and therefore likely reflects a play of chance. Regardless, adjusting the analyses for hepatitis status (or excluding hepatitis cases) did not significantly alter the associations between *SLC30A10* genotype and biochemical liver markers or risk of liver disease.

In conclusion, a rare missense variant in the manganese transporter *SLC30A10* is associated with elevated alanine transaminase, and with increased MRI-quantified hepatic inflammation.

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Statements & Declarations

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Animal Research (Ethics)

Not applicable.

Consent to Participate (Ethics)

All participants in the Copenhagen cohort and UK Biobank provided written consent.

Consent to Publish (Ethics)

All co-authors agreed to the final version of the manuscript, and to the decision to submit for publication.

Plant reproducibility

Not applicable.

Clinical Trials Registration

No applicable.

Author Contributions

A.S.: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. B.G.N.: Resources, Writing – review & editing. A.T.H.: Resources, Writing – review & editing. H.Y.: Resources, Formal analysis, Writing – review & editing. S.S.: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. All authors approved the final version of the manuscript.

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

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Figure legends

Graphical abstract. A rare genetic variant (p.Thr95Ile) in the manganese transporter *SLC30A10* is associated with elevated plasma alanine transaminase (ALT) and higher corrected T1 on liver MRI, markers of liver inflammation. These data support that the variant may increase the risk of liver disease.

Figure 1. Density plots of plasma alanine transaminase in UKB stratified by *SLC30A10* p.Thr95Ile and *PNPLA3* p.Ile148Met. The vertical dashed lines depict medians for the respective genotypes.