Genetic studies of MRI liver iron content identify susceptibility loci and yield insights into its link with other diseases.

Henry R. Wilman*&, Constantinos A. Parisinos*&, Naeimeh Atabaki-Pasdar, Matt Kelly, E Louise Thomas, Stefan Neubauer, IMI DIRECT Consortium, Anubha Mahajan, Aroon D. Hingorani, Riyaz S. Patel, Harry Hemingway, Paul W. Franks, Jimmy D. Bell, Rajarshi Banerjee, Hanieh Yaghootkar&

*** Joint first authors & These authors contributed equally**
Corresponding authors:
1) Dr Constantinos Parisinos (Email: c.parisinos@ucl.ac.uk) Institute of Health Informatics, Faculty of Population Health Sciences, University College London, NW12DA Phone number: +(44)7899786998
2) Dr.Hanieh Yaghootkar (Email: h.yaghootkar@exeter.ac.uk). College of Medicine and Health, RILD building Level 3, Royal Devon & Exeter Hospital, Barrack Road, Exeter, EX2 5DW) Phone number +(44)7576890854

Affiliations

H.R.W: Research Centre for Optimal Health, School of Life Sciences, University of Westminster, London, U.K. and Perspectum Diagnostics Ltd., Oxford, UK - h.wilman@westminster.ac.uk
C.A.P: Institute of Health Informatics, Faculty of Population Health Sciences, University College London, London, UK - c.parisinos@ucl.ac.uk
N.A: Department of Clinical Sciences, Genetic and Molecular Epidemiology Unit, Lund University, Skåne University Hospital Malmö, Malmö, Sweden. naimeh.atabaki_pasdar@med.lu.se
M.K: Perspectum Diagnostics Ltd., Oxford, UK - matt.kelly@perspectum-diagnostics.com
E.L.T: Research Centre for Optimal Health, School of Life Sciences, University of Westminster, London, U. - l.thomas3@westminster.ac.uk
S.N: Oxford Centre for Clinical Magnetic Resonance Research, Division of Cardiovascular Medicine, Oxford NIHR Biomedical Research Centre, University of Oxford, Oxford, UK and Perspectum Diagnostics Ltd., Oxford, UK - stefan.neubauer@perspectum.com
IHI Direct Consortium: info@direct-diabetes.org
A.M: Wellcome Centre for Human Genetics, University of Oxford, Oxford, U.K. - anubha@well.ox.ac.uk
A.D.H: Institute of Cardiovascular Science, Faculty of Population Health Sciences, University College London, London, UK - a.hingorani@ucl.ac.uk
R.S.P: Institute of Cardiovascular Science, Faculty of Population Health Sciences, University College London, London, UK - riyaz.patel@ucl.ac.uk
H.H: Health Data Research UK London, Institute of Health Informatics, Faculty of Population Health Sciences, University College London, London, UK - h.hemingway@ucl.ac.uk
P.W.F: Department of Clinical Sciences, Genetic and Molecular Epidemiology Unit, Lund University, Skåne University Hospital Malmö, Malmö, Sweden. Department of Public Health & Clinical Medicine, Umeå University, Umeå, Sweden. - paul.franks@med.lu.se
J.D.B: Research Centre for Optimal Health, School of Life Sciences, University of Westminster, London, UK - j.bell@westminster.ac.uk
R.B: Perspectum Diagnostics Ltd., Oxford, UK rajarshi.banerjee@perspectum-diagnostics.com
H.Y: Genetics of Complex Traits, College of Medicine and Health, University of Exeter, Exeter, UK. and Research Centre for Optimal Health, School of Life Sciences, University of Westminster, London, UK h.yaghootkar@exeter.ac.uk

Keywords: Magnetic resonance imaging, iron, metabolism, metabolic syndrome, genome-wide association study, genetics

Ethical approval This research has been conducted using data from the UK Biobank resource and carried out under UK Biobank project application numbers 9914 and 31037. UK Biobank protocols were approved by the National Research Ethics Service Committee.

Patient consent No participants were directly involved in our study, as we used data derived from the UK Biobank study, under project application numbers 9914 and 31037. For the UK Biobank overall study, participants signed written informed consent, specifically applicable to health-related research. All ethical regulations were followed. No patients or participants were specifically or directly involved in setting the research question or the outcome measures or in developing plans for recruitment, design, or implementation of this study. No patients were asked to advise on interpretation or drafting of results. There are no specific plans to disseminate the research results to study participants, but the UK Biobank disseminates key findings from projects on its website.

Word Count: 5178

Number of figures and tables (excluding Supplementary Material): 5 Figures, 2 Tables

Number of figures and tables (Supplementary Material Only): 10 Supplementary Figures, 11 Supplementary Tables

Financial Support Statement: H.Y. is funded by a Diabetes UK RD Lawrence fellowship (17/0005594). C.A.P is funded by a Wellcome Trust Clinical PhD Programme (206274/Z/17/Z). H.W is funded by an Innovate UK Knowledge Transfer Partnership (KTP10271). The work undertaken by P.W.F was supported in part by ERC-2015-CoG_NASCENT_681742. IMI-DIRECT was supported by the Innovative Medicines Initiative Joint Undertaking under grant agreement n°115317 (DIRECT), resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in-kind contribution. H.H is a National Institute for Health Research (NIHR Senior Investigator).

Conflict of Interest statement: M.K and R.B. are employees and shareholders of Perspectum Diagnostics. H.W. and S.N. are shareholders in Perspectum Diagnostics. The IMI DIRECT Study was funded in part by Boehringer Ingelheim, Eli Lilly, Novo Nordisk, Sanofi Aventis, and Servier. No other potential conflicts of interest relevant to this article were reported.

Authors contributions H.W, C.A.P, H.Y were involved in the study conception and design, analysis and writing of the manuscript. H.H, A.D.H, R.S.P edited the manuscript and supervised C.A.P. E.L.T, JB, edited the manuscript and wrote elements of the discussion. S.N, R.B, M.K, edited the manuscript and provided the infrastructure underlying the MRI liver iron content measurements. P.W.F, A.M, N.A contributed to the analysis of the validation cohort (IMI DIRECT).

ABSTRACT

Background & Aims: Excess liver iron content is common and is linked to hepatic and extrahepatic disease risk. We aimed to identify genetic variants influencing liver iron content and use genetics to understand its link to other traits and diseases.

Methods: First, we performed a genome-wide association study (GWAS) in 8,289 individuals in UK Biobank with MRI quantified liver iron, and validated our findings in an independent cohort (n=1,513 from IMI DIRECT). Second, we used Mendelian randomisation to test the causal effects of 29 predominantly metabolic traits on liver iron content. Third, we tested phenome-wide associations between liver iron variants and 770 anthropometric traits and diseases.

Results: We identified three independent genetic variants (rs1800562 (C282Y) and rs1799945 (H63D) in *HFE* and rs855791 (V736A) in *TMPRSS6*) associated with liver iron content that reached the GWAS significance threshold ($p<5x10^{-8}$). The two *HFE* variants account for ~85% of all cases of hereditary haemochromatosis. Mendelian randomisation analysis provided evidence that higher central obesity plays a causal role in increased liver iron content. Phenome-wide association analysis demonstrated shared aetiopathogenic mechanisms for elevated liver iron, high blood pressure, cirrhosis, malignancies, neuropsychiatric and rheumatological conditions, while also highlighting inverse associations with anaemias, lipidaemias and ischaemic heart disease.

Conclusion: Our study provides genetic evidence that mechanisms underlying higher liver iron content are likely systemic rather than organ specific, that higher central obesity is causally associated with higher liver iron, and that liver iron shares common aetiology with multiple metabolic and non-metabolic diseases.

Lay summary: Excess liver iron content is common and is associated with liver diseases and metabolic diseases including diabetes, high blood pressure, and heart disease. We find that three genetic variants are linked to increased risk of developing higher liver iron content. We show that the same genetic variants are linked to higher risk of many diseases, but they may also be associated with some health advantages. Finally, we use genetic variants associated with waist-to-hip ratio as a tool to show that central obesity is causally associated with increased liver iron content.

INTRODUCTION

Liver disease constitutes the third most common cause of premature death in the UK, and its prevalence is substantially higher compared to other countries in Western Europe.[1–3] Excess liver iron is associated with increased severity and progression of liver diseases including cirrhosis and hepatocellular carcinoma in individuals with non-alcoholic fatty liver disease (NAFLD), [4–6] and is the direct cause of liver disease in those with hereditary haemochromatosis and thalassaemia.[7,8] Observational associations have been described between excess liver iron content and several metabolic diseases such as high blood pressure, obesity, polycystic ovarian syndrome and type 2 diabetes - a condition recognised as dysmetabolic iron overload syndrome (DIOS) which affects up to 5-10% of the general population.[9,10]

The associations between excess liver iron and hepatic and non-hepatic diseases necessitate exploration of underlying pathophysiological mechanisms. There is little known about the genetic background of liver iron content. Studies of hereditary haemochromatosis patients with autosomal recessive mutations show they have higher liver iron, measured from biopsies, when compared to controls, however no studies have been performed in unselected populations. Furthermore, it is unknown whether iron accumulation is a systemic disorder involving multiple organs or whether there are mechanisms specific to the liver. Previous genome-wide association studies (GWAS) have focussed on peripheral biochemical markers of iron status that do not correlate well with liver iron.[11]

Measuring liver iron has traditionally been difficult. Liver biopsy, the “gold standard” for assessment of liver iron, is an invasive procedure and therefore unsuitable for population research studies. An alternative is magnetic resonance imaging (MRI); a non-invasive,

quick, robust and validated method for quantifying liver iron content.[12] The availability of genetic and clinical data, as well as MRI scans of liver in the UK Biobank cohort has provided an unparalleled opportunity to study the genetics of liver iron content in the general population.

The aim of this study was to (i) identify genetic variants specifically associated with liver iron content, (ii) investigate which metabolic traits and diseases might cause higher liver iron content, and (iii) characterise the traits/diseases associated with liver iron content susceptibility variants. To facilitate this, we performed the first GWAS of MRI-determined liver iron content in 8,289 UK Biobank participants and replicated our findings in an independent cohort of 1,513 participants of European ancestry from the IMI DIRECT study (**Figure 1**).[13,14]

METHODS

UK Biobank participants

UK Biobank consists of over 500,000 individuals aged 37–73 years (99.5% were between 40 and 69 years of age) who were recruited between 2006 and 2010 from across the U.K.[13] This research has been conducted using the data obtained via UK Biobank Access Application number 9914. UK Biobank field numbers used for this analysis can be found in **Supplementary Table 1**. We used data from the first subset of UK Biobank participants invited for multiparametric MRI imaging between 2014 and 2016.[15] After image analysis and quality control steps (see below), liver iron was available for 8,674 individuals who also had genetic data. We based our study on 8,289 individuals of white European descent as defined by principal component (PC) analysis. Briefly, we first generated PCs in the 1000 Genomes cohort using high-confidence SNPs to obtain their individual loadings. We then used these loadings to project all the UK Biobank samples into the same PC space, and individuals were clustered using PCs 1–4.

Genetic Data

Protocols for the participant genotyping, data collection, and quality control have previously been described in detail.[13] Briefly, participants were genotyped using one of two purpose-designed arrays (UK BiLEVE Axiom Array (n= 50,520) and UK Biobank Axiom Array (n = 438,692)) with 95% marker overlap. We excluded individuals who were identified by the UK Biobank as outliers based on either genotyping missingness rate or heterogeneity, or whose sex inferred from the genotypes did not match their self-reported sex. We removed individuals with a missingness >5% across variants which passed our quality control procedure. We used the latest release which included imputed data using

two reference panels: a combined UK10K and 1000 Genomes panel and the Haplotype Reference Consortium (HRC) panel. We limited our analysis to genetic variants with a minimum minor allele frequency (MAF) > 1% and imputation quality score (INFO) > 0.3.

Imaging protocol and analysis

The imaging protocol and analysis of liver iron content in UK Biobank participants has previously been published.[15] Briefly, participants were scanned at the UK Biobank centre in Cheadle (UK) using a Siemens 1.5T Magnetom Aera. A single-breath-hold MRI sequence was acquired as a single transverse slice captured through the centre of the liver, superior to the porta hepatis. This sequence forms part of the UK Biobank abdominal imaging protocol. The data was analysed using the Liver*MultiScan*™. Discover software by a team of trained analysts, blinded to any subject variables. Analysts selected three 15mm diameter circular regions of interests, to cover a representative sample of the liver parenchyma, avoiding vessels, bile ducts and other organs. The repeatability and reproducibility of the image analysis was high.[15]

Genome-wide association analysis

We performed the association tests using 2 different software: (1) GEMMA version 0.96 as our main analysis using all individuals of genetically defined Europeans (n=8,289),[16] and (2) PLINK version 1.9 as our sensitivity analysis using unrelated white British individuals (defined in UK Biobank field 22006, n=6,758).[17]

GEMMA applies a linear mixed-model (LMM) to adjust for the effects of population structure and relatedness. Therefore, we increased our power by including all related individuals of European descent. The relatedness matrix was computed using common (MAF>5%) genotyped variants that passed quality control. Prior to association testing,

liver iron was first log-transformed and then adjusted for age, sex and study centre and in our sensitivity analysis additionally for BMI or alcohol consumption. We then inverse normal transformed the values. At runtime, we included genotyping array (as a categorical variable for UKBileve array, UKB Axiom array interim release and UKB Axiom array full release) as a covariate.

We used PLINK to perform a sensitivity analysis. Prior to association testing, liver iron was adjusted for age, sex, BMI and genotyping array, and then we quantile normalised the resulting values. At runtime, we included the first 10 genetic PCs (UK Biobank field 22009) as covariates to control for confounding by population stratification. We used Quanto (http://biostats.usc.edu/Quanto.html) to calculate our discovery GWAS power in 8,289 individuals from UK Biobank at different allele frequencies and effect sizes at α level 5×10^{-8} assuming additive effect model (**Supplementary Figure 1**).

LD Score regression and cross-trait genetic correlation analysis

We used LDHub to conduct linkage disequilibrium (LD) score regression and heritability analysis. LD Hub is a centralized database of summary level GWAS for > 100 diseases/traits from publicly available resources/consortia and uses a web interface that automates LD score regression, heritability and cross-trait genetic correlation analysis pipeline.[18] We ran heritability analysis as well as genetic correlation analysis across 448 potentially relevant traits. SNP-based heritability (h^2_{SNP}) is the proportion of total variation in liver iron content due to the additive genetic variation between individuals in our study population.

Gene-set and tissue expression enrichment analysis

We performed gene-set and tissue expression analyses using MAGMA.[19] Lead variants were assigned to a minimum P of $5x10^{-8}$. We used the default settings provided by the software. We chose 1000 Genomes Phase 3 as the reference panel population. The minimum minor allele frequency was set to 0.01. We used a maximum allowed distance of 250k between LD blocks for variants to be included in the same locus.

For gene-set enrichment analyses, positional mapping was used with variants assigned to a gene if they were within the gene start and end points (by setting the distance either side to 0kb). Only protein-coding genes were included in the mapping process. Tested gene-sets include BioCarta, REACTOME, KEGG and GO. Bonferroni correction was used to adjust for the number of gene-sets tested. Analysis of differentially expressed genes were based on data from GTEx v6 RNA-seq data.[20]

Replication analysis

Associations reaching $P < 5x10^{-8}$ were followed up in the IMI DIRECT cohort. IMI DIRECT includes 1,513 participants who had both liver iron and GWAS data to replicate our findings. The IMI-DIRECT consortium is a collaboration among investigators from a range of European academic institutions and pharmaceutical companies.[14] Liver iron was measured using a T2*-based multiecho MRI technique.[21] DNA extraction was carried out using Maxwell 16 Blood DNA purification kits and a Maxwell 16 semi-automated nucleic acid purification system (Promega). Genotyping was conducted using the Illumina HumanCore array (HCE24 v1.0) and genotypes were called using Illumina's GenCall algorithm. A total of 517,958 markers passed quality control procedures. We took autosomal variants with MAF>1% that passed quality control and constructed axes of genetic variation using PC analysis implemented in the GCTA software to identify ethnic

outliers defined as non-European ancestry using the 1000 Genomes samples as reference. We identified six individuals as ethnic outliers.

We performed the association tests in 3 models: (i) non-diabetic participants (n=1,010), (ii) diabetic participants (n=503), and (iii) combined (n=1,513). We took residuals from a model of liver iron and age, sex, BMI, 10 PCs and Centres and then inverse-normal transformed the values.

Sensitivity Analyses

We performed 4 sensitivity analyses. First, to assess whether there is any sex-specific association, we carried out GWASs in men and women separately. Second, to test whether relatedness was responsible for any of the individual variant associations and replicate GEMMA's results, we ran a GWAS using only unrelated Europeans individuals in UK Biobank and a different GWAS software tool (PLINK version 1.9). Third, we adjusted models for alcohol intake frequency (field 1558; categories treated as ordinal scale – "Never"=0 to "Daily or almost daily"=5) measured at baseline that may have had an impact on liver iron. Individuals responding "Do not know" or "Prefer not to answer" for "Alcohol intake frequency" were excluded from this sensitivity analysis. Fourth, to investigate the potential for collider bias resulting from conditioning liver iron on BMI, we performed a GWAS of liver iron without adjustment for BMI.

Mendelian randomisation

Multiple traits have shown association with liver iron in observational studies, including BMI, lipids and non-alcoholic fatty liver disease.[10,22–24] We therefore investigated the causal effects of 29 predominantly metabolic traits using two-sample Mendelian randomisation analysis.[25] Mendelian randomisation is a method that uses genetic

variants associated with the exposure (e.g. metabolic traits) to infer causal relationships between an exposure and an outcome (e.g. liver iron content). This method relies on a simple principle; if a modifiable exposure is causal for a disease, then the genetic variants associated with that exposure will also be associated with disease risk. Since genetic variants are inherited at birth, Mendelian randomisation experiments are free from confounding and biases that are seen in observational studies.

Following correction for multiple testing, associations with a false discovery rate (FDR) <5% were considered statistically significant. We used the inverse variance weighted approach (IVW) as our main analysis, and MR-Egger and penalised weighted median as sensitivity analyses in the event of unidentified pleiotropy of our genetic instruments. Genetic instruments for the 29 metabolic traits as an exposure were constructed by developing risk scores using only genome-wide significant SNPs that were not in linkage disequilibrium (R^2 <0.1).[26–28]

Phenome-wide association study (PheWAS)

We used the SNPs associated with liver iron content and carried out a PheWAS using publicly available summary statistics from GWASs on predefined ICD10 disease codes, anthropometric traits, and self-reported conditions previously carried out in 452,264 UK Biobank participants of European ancestry,[29] as well as publicly available, curated summary statistics from previous GWAS (**Supplementary Tables 2-4**).[30] Associations with a false discovery rate (FDR) <5% were considered statistically significant. A description of how ICD codes were grouped to represent a clinical phenotype are provided elsewhere.[29]

RESULTS

The characteristics of liver iron content cohort.

The median liver iron content in UK Biobank was 1.28 mg/g (interquartile range (IQR):1.16-1.44 mg/g) in men (n=3,928) and 1.23 mg/g (IQR: 1.13-1.38) in women (n=4,361) (**Table 1, Supplementary Figure 2**). Among men 6.5% and among women 3.4% had an elevated liver iron content, above the commonly accepted 1.8mg/g threshold.[31] In IMI DIRECT cohort, the median liver iron content was 1.3 (1.2-1.5) in both men (n=1,101) and women (n=412). BMI, waist circumference and diabetes prevalence were lower in the liver iron cohort (n=8,289) than the remainder of UK Biobank (n=402,071) (**Supplementary Table 5**). Although invitation was not based on any medical information, MRI exclusion criteria (e.g. metal or electrical implants, surgery in six weeks prior to appointment, severe hearing or breathing problems) may have also contributed to a slightly healthier cohort. The Townsend deprivation index was on average lower in this study cohort. This may be related to MRI imaging participants being biased towards those who live close to the imaging center (Cheadle) where all of the liver iron cohort were imaged.

There are three genetic variants associated with liver iron content.

We performed a GWAS of MRI derived measures of liver iron using 8,289 individuals of European ancestry from UK Biobank (**Figure 2, Supplementary Figure 3)**. We estimated to have more than 80% power in our discovery set to detect variants with MAF>=5% and effect size >= 0.2 standard deviation (SD) on liver iron content (**Supplementary Figure 1**). We detected no evidence for inflation of test statistics (λ_{GC} = 1.016). Our discovery GWAS identified 4 independent variants at $P<5x10^{-8}$ (**Table 2**). Two independent variants

lie within *HFE*: C282Y (rs1800562; 0.41 SD increase in liver iron per allele; p=5.2x10^{-42}) and H63D (rs1799945; 0.17 SD; p=8.2x10^{-15}). The third variant lies in *TMPRSS6*; V736A (rs855791; 0.11 SD; p=1.3x10^{-11}). The fourth variant, rs149275125, lies between *HS3ST3B1* and *PMP22* (0.41 SD; p=3x10^{-9}).

In 1,513 IMI DIRECT participants, we replicated all three common variants at $p < 4x10^{-4}$ with a consistent direction of effect and similar effect size **(Supplementary Table 6)**. The rare variant did not associate with liver iron in IMI DIRECT and the direction of effect was opposite to our discovery dataset. This variant is a rare variant (MAF 1%) and has not previously been reported to be associated with any other traits. We focused all other analyses on the three replicated variants.

Both *HFE* and *TMPRSS6* produce proteins that form part of the signalling pathway regulating hepcidin production, the key hormone responsible for iron balance in the body. C282Y homozygotes and C282Y/H63D compound heterozygotes account for ~85% of cases of hereditary haemochromatosis.[7] In UK Biobank, 35 individuals (0.4%) were C282Y homozygotes and had the highest levels of liver iron (mean: 2.39 mg/g (±1.2)); 182 (2.2%) were C282Y/H63D compound heterozygotes (1.75 mg/g (±0.7)); 186 (2.2%) were H63D homozygotes (1.46 mg/g (±0.47)); 2,920 (35%) were either C282Y or H63D heterozygotes (1.34 mg/g (±0.32)) and 4,966 (60%) did not have any of the variants and had the lowest liver iron (1.28 mg/g (±0.24)) (**Figure 3**).

Correlation between the effect sizes and p-values in the two separate GWASs carried out in GEMMA and PLINK showed strong agreement (**Supplementary Figures 4, 5**). We did not detect any sex-specific variants and the magnitude of effect was similar between men and women (**Supplementary Table 6, Supplementary Figure 6**). The sensitivity

analysis adjusting for alcohol consumption and BMI did not identify any additional signal and did not change the effect size (**Supplementary Table 6**). Our pathway analysis demonstrates overlap between liver iron gene-sets and pathways involved in autism and schizophrenia (**Supplementary Figure 7**). Nearby genes were visualised with locuszoom plots (**Supplementary Figures 8, 9**)

Liver iron content is heritable and has a high genetic correlation with blood levels of iron biomarkers.

We estimated the SNP-based heritability (h^2_{SNP}) of liver iron to be 7%. This is similar to heritability estimated for conditions and traits such as coronary artery disease (7%)[32] and eczema (7%),[33] but lower than heritability estimated for body fat % (10%)[34] and transferrin (16%).[11]

To identify genetic overlap between liver iron content and other diseases and traits, we performed LD score regression analyses against a range of available traits and diseases with GWAS summary statistics (448 traits/diseases, **Supplementary Table 7**). The most genetically correlated traits were transferrin (r_G=-0.78, *P*=0.04) and ferritin (r_G=1.24, *P*=0.05) with nominal significant correlation. Joint disorders (r_G=-1.17, *P*=0.50), hypertrophic cardiomyopathy (r_G=-1.11, *P*=0.35), type 2 diabetes (r_G=0.44, *P*=0.17), chronic kidney disease (r_G=0.57, *P*=0.48), tinnitus (r_G=0.65, *P*=0.17), polyuria (r_G=0.75, *P*=0.36), and gout (r_G=0.90, *P*=0.19) were highly correlated (r_G>0.4) but did not reach a nominal significance threshold (*p*>0.05). Metabolic traits including fasting insulin (r_G=0.17, *P*=0.53), HOMA-IR (r_G=0.37, *P*=0.48), fasting glucose (r_G=0.01, *P*=0.97) and coronary artery disease (r_G=-0.01, *P*=0.97) were not genetically correlated with liver iron content.

Gene-set enrichment analysis did not identify any enriched tissue or pathways.

We used MAGMA implemented as part of the FUMA GWAS platform to assess tissue enrichment of genes at associated loci. We did not find any tissue enrichment, but differentially expressed gene sets were enriched in blood vessels, lung, and adipose tissue, although they did not reach a significant threshold following adjustment for multiple testing (**Supplementary Table 8**). None of the pathways reached our FDR significance threshold (**Supplementary Table 9)**.

Mendelian randomisation analysis provides evidence for a causal link between central obesity and liver iron content.

We examined the potential causal effect of 29 metabolic traits and diseases **(Figure 4, Supplementary Table 10)** on liver iron content. Following correction for multiple testing (FDR<5%), we found evidence of a causative effect of central obesity, as measured by higher waist-to-hip ratio (adjusted for BMI), on elevated liver iron content (IVW *P*=0.003) **(Supplementary Table 9, Supplementary Figure 10)**. There was suggestive evidence that higher fasting glucose (IVW *P*=0.03), higher NAFLD (IVW *P*=0.04) and higher alanine transaminase (IVW *P*=0.05) were causally associated with higher liver iron content but none of these associations reached our multiple testing threshold of being statistically significant. All the results were robust to a range of Mendelian randomisation sensitivity analyses.

As a positive control, elevated transferrin saturation levels (IVW *P*=0.0007), blood iron content (IVW *P*=0.01) and ferritin levels (IVW P=0.01) were causally associated with higher liver iron content. The genetic variants (between 5 to 6 variants) associated with these serum iron parameters include the 3 variants associated with liver iron content (*HFE* C282Y, *HFE* H63D, *TMPRSS6* V736A). Therefore, it was not possible to test the causal

effect of these parameters on liver iron content independent of *HFE* and *TMPRSS6* genetic variants.

PheWAS identifies novel associations of liver iron variants with traits and diseases.

The three liver iron content variants had been previously reported to be associated with multiple haematological parameters, glycated haemoglobin, lipid and bilirubin levels, as well as blood pressure traits (**Supplementary Table 11**). We performed a hypothesis-free PheWAS to investigate the association of these three variants with other traits and diseases using predefined ICD10 codes, self-reported conditions and traits from UK Biobank and publicly available GWAS.

HFE C282Y was associated with higher liver fibrosis/cirrhosis, higher risk of type 2 diabetes, hypertension, alcoholic liver disease, arthrosis, chronic and degenerative neurological problem including multiple sclerosis, arthritis and higher height but lower total cholesterol, lower LDL-C and lower BMI (FDR<5%, **Figure 5, Supplementary Table 2**). *HFE* H63D was associated with higher risk of hypertension, ankylosing spondylitis and bladder malignancy but lower risk of malabsorption or coeliac disease and lower cognitive ability (FDR<5%, **Figure 5, Supplementary Table 3**). The liver iron increasing allele at *TMPRSS6* was associated with lower risk of ischaemic heart disease, angina pectoris, and lipidaemias (FDR<5%, **Figure 5, Supplementary Table 4**).

DISCUSSION

We performed the first GWAS of multi-parametric MRI determined liver iron content in an unselected population. The identification of loci implicated in increased iron absorption (*HFE* and *TMPRSS6*) provides genetic validation of the utility of MRI for the non-invasive assessment of liver iron content.

The three independent variants in *HFE* and *TMPRSS6* have previously been reported to be associated with circulating iron traits including transferrin saturation, blood iron, ferritin, and transferrin levels.[11] Both *HFE* and *TMPRSS6* play a major role in iron homeostasis by modulating the expression of hepcidin production by the liver.[35],[36] Hepcidin inhibits iron transport and absorption from the gut into the circulation by binding to the main iron transport channel expressed on the surface on duodenal enterocytes, ferroportin.[37] *TMPRSS6* encodes matriptase 2 (MTP-2), a liver serine protease, which inhibits hepcidin leading to higher iron absorption and bioavailability. *In vitro* studies have shown that major allele at rs888571 inhibits hepcidin more effectively than missense variant rs888571(V736A).[38] *HFE* is a positive upstream regulator of hepcidin transcription. Missense variants C282Y and H63D in *HFE* result in lower hepcidin responsiveness to iron, leading to relative or absolute hepcidin deficiency and subsequent increase in iron absorption and bioavailability.[39]

Elevated liver iron is observationally associated with multiple metabolic traits and diseases in a common condition described as DIOS.[10] Our Mendelian randomisation analysis supports a causal role for higher central obesity on higher liver iron content, providing further evidence for DIOS. Other traits such as fasting glucose, NAFLD, and alanine transaminase showed suggestive causal associations. Animal studies have suggested a putative mechanism through defective iron handling and subsequent iron

overload due to an inflammatory shift and cytokine secretion by activated macrophages that accumulate around adipocytes in obesity.[40,41] The underlying mechanism, however, is still unclear, and is likely to involve a complex interplay between diet and genetic factors as well as cross-talk between liver and visceral adipose tissue.[10]

Our GWAS study identified variants that are likely to regulate iron stores systemically and are not specific to the liver. We, therefore, were not able to examine the causal role of higher liver iron content *per se* on other diseases and traits using Mendelian randomisation. To investigate, however, the phenotypic architecture and shared pathological mechanisms of higher liver iron content with other traits and diseases, we carried out a PheWAS of the 3 genome-wide significant variants against all available disease outcomes and traits from UK Biobank[29] and publicly available genetic summary statistics.[30]

Our PheWAS indicates *HFE* C282Y is associated with arthrosis, coxarthrosis, osteoarthritis, and gout, and *HFE* H63D is associated with ankylosing spondylitis and has a suggestive association with dorsalgia. The association between *HFE* C282Y and higher risk of cellulitis, abscesses, furuncles and curbuncles, subcutaneous infections as well as osteomyelitis provides further evidence that genetically elevated iron levels are associated with higher infection risk. Some infectious disease agents are more virulent in an environment with excess iron. There is also evidence that iron overload compromises the ability of phagocytes to kill microorganisms.[42]

Higher iron is correlated with carcinogenesis.[43] An important mechanism may be oxidative stress and the catalytic activity of iron in the formation of hydroxyl radicals. Iron may also suppress host defence cell activity and promote cancer cell proliferation. A recent study found an association between *HFE* C282Y and higher risk of breast cancer,

colorectal cancer, hepatocellular carcinoma, and total cancer risk.[44] We found additional evidence of associations with extra-hepatic malignancies including bladder cancer (OR=1.0004 per copy of H63D, p=4.7x10^{-6}, FDR 0.03) and renal cancer (OR=1.0005 per copy of C282Y, p=0.004, FDR 0.05). Despite very small effects, these findings provide genetic evidence for shared mechanisms underlying higher liver iron and extra-hepatic cancers.

The association between *HFE* C282Y and neurological conditions such as multiple sclerosis and epilepsy is consistent with the role of iron in many important processes in the central nervous system, including oxygen transportation, oxidative phosphorylation, myelin production, and the synthesis and metabolism of neurotransmitters. In a recent GWAS of brain MRI scans, *HFE* C282Y was associated with iron accumulation in certain parts of the brain.[45] Observational studies show that iron accumulation in the brain is associated with multiple sclerosis, parkinsonism and Alzheimer's disease.[46,47] Individuals with hereditary haemochromatosis frequently develop psychological symptoms, including extreme fatigue, irritability and depression.[48] Our pathway analysis demonstrates overlap with gene-sets and pathways involved in autism and schizophrenia. *HFE* H63D was associated with a reduction in reaction time in specific cognitive function tests, providing further evidence that iron accumulation may cause premature, and indeed preventable, cognitive decline.

The association between *HFE* variants and hypertension is consistent with previous findings[49] Possible mechanisms include increased vascular tone secondary to the generation of reactive oxygen species and oxidative stress,[50] or excess iron accumulation in renal arterioles leading to activation of the renin-angiotensin aldosterone

system. We further validated the known association between *HFE* C282Y and type 2 diabetes which could be, at least partly, due to iron accumulation in the pancreas.

The liver iron increasing allele at *TMPRSS6* rs855791 was associated with lower LDL-C, lower risk of angina and ischaemic heart disease. Similar observations have been reported in *HFE* C282Y homozygotes.[51] A Mendelian randomisation study has recently reported that elevated circulating iron may have a causal (protective) effect on coronary artery disease.[52] The underlying mechanism is unclear. It is possible that part of this effect is driven through effects on haematological parameters, or lower LDL-C. In conditions where excess iron stores are treated (e.g. hereditary haemochromatosis), further research is needed on whether LDL-C levels subsequently increase, and whether the risk of coronary artery disease can be kept low with statins and other preventive interventions.[51]

This study is limited in that the UK Biobank MRI cohort is not a completely unbiased sample of the population. The UK Biobank MRI cohort is slightly more healthy, wealthy, and well educated compared to the whole cohort of 40-69 year olds in the UK.[53] The population studied in this work has a slightly lower average BMI and waist circumference than the UK Biobank population as a whole. Larger GWAS studies (e.g. on completion of the full 100,000 UK Biobank imaging cohort) may elucidate further susceptibility loci. Ongoing development and validation of MRI scores that may allow accurate determination of the level of inflammation and fibrosis, may lead to further genetic studies focussing on the more severe spectrum of liver disease.

Conclusion

We report a large GWAS for MRI liver iron content and identify 3 susceptibility loci previously implicated in circulating iron traits. We provide genetic validation for multi-parametric MRI as a novel, non-invasive and radiation free imaging modality for liver iron content. Our genetic study suggests that higher liver iron content may be caused in part by higher central adiposity. The deposition of excess iron in the liver seems to share common mechanisms with circulating iron accumulation, which eventually results in widespread damage to parenchymal tissue, leading to several pathologies through a common mechanism.

Acknowledgments

This research has been conducted using the UK Biobank resource and carried out under UK Biobank project numbers 9914 and 31037. Details on patient and public involvement in the UK Biobank are available online at www.ukbiobank.ac.uk/about-biobank-uk and www.ukbiobank.ac.uk/wp-content/uploads/2011/07/Summary-EGF-consultation.pdf. No patients were specifically involved in setting the research question or the outcome measures or in developing plans for recruitment, design, or implementation of this study. No patients were asked to advise on interpretation or drafting of results. There are no specific plans to disseminate the research results to study participants, but the UK Biobank disseminates key findings from projects on its website. H.Y. is funded by a Diabetes UK RD Lawrence fellowship (17/0005594). C.A.P is funded by a Wellcome Trust Clinical PhD Programme (206274/Z/17/Z). H.W is funded by an Innovate UK Knowledge Transfer Partnership (KTP10271). The work undertaken by P.W.F was supported in part by ERC-2015-CoG_NASCENT_681742. IMI-DIRECT was supported by the Innovative Medicines Initiative Joint Undertaking under grant agreement n°115317 (DIRECT), resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in-kind contribution. H.H is a National Institute for Health Research (NIHR Senior Investigator).

Data sharing

Full summary statistics of the MRI liver iron genome-wide association study will be publically available via UK Biobank.

[1] Williams R, Aspinall R, Bellis M, Camps-Walsh G, Cramp M, Dhawan A, et al. The Lancet Commissions Addressing liver disease in the UK: a blueprint for attaining excellence in health care and reducing premature mortality from lifestyle issues of excess consumption of alcohol, obesity, and viral hepatitis The Lancet Commissions. 2014;Available from: http://dx.doi.org/10.1016/

[2] Rinella ME. Nonalcoholic fatty liver disease: a systematic review. JAMA. 2015;313:2263–2273.

[3] Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64:73–84.

[4] Pais R, Charlotte F, Fedchuk L, Bedossa P, Lebray P, Poynard T, et al. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. J. Hepatol. 2013;59:550–556.

[5] Valenti L, Fracanzani AL, Bugianesi E, Dongiovanni P, Galmozzi E, Vanni E, et al. HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology. 2010;138:905–912.

[6] Sorrentino P, D'Angelo S, Ferbo U, Micheli P, Bracigliano A, Vecchione R. Liver iron excess in patients with hepatocellular carcinoma developed on non-alcoholic steato-hepatitis. J. Hepatol. 2009;50:351–357.

[7] Mohamed M, Phillips J. Hereditary haemochromatosis. BMJ. 2016;353:i3128.

[8] Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. Lancet. 2018;391:155–167.

[9] Ehrmann DA. Polycystic ovary syndrome. N. Engl. J. Med. 2005;352:1223–1236.

[10] Deugnier Y, Bardou-Jacquet É, Lainé F. Dysmetabolic iron overload syndrome (DIOS). Presse Med. 2017;46:e306–e311.

[11] Benyamin B, Esko T, Ried JS, Radhakrishnan A, Vermeulen SH, Traglia M, et al. Novel loci affecting iron homeostasis and their effects in individuals at risk for hemochromatosis. Nat. Commun. 2014;5:4926.

[12] Gandon Y, Olivié D, Guyader D, Aubé C, Oberti F, Sebille V, et al. Non-invasive assessment of hepatic iron stores by MRI. Lancet. 2004;363:357–362.

[13] Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. PLoS Med. 2015;12:e1001779.

[14] Koivula RW, Heggie A, Barnett A, Cederberg H, Hansen TH, Koopman AD, et al. Discovery of biomarkers for glycaemic deterioration before and after the onset of type 2 diabetes: rationale and design of the epidemiological studies within the IMI DIRECT Consortium. Diabetologia. 2014;57:1132–1142.

[15] McKay A, Wilman HR, Dennis A, Kelly M, Gyngell ML, Neubauer S, et al. Measurement of liver iron by magnetic resonance imaging in the UK Biobank population. PLoS One. 2018;13:e0209340.

[16] Zhou X, Stephens M. Genome-wide efficient mixed-model analysis for association studies. Nat. Genet. 2012;44:821–824.

[17] Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience. 2015;4:7.

[18] Zheng J, Erzurumluoglu AM, Elsworth BL, Kemp JP, Howe L, Haycock PC, et al. LD Hub: a centralized database and web interface to perform LD score regression that maximizes the potential of summary level GWAS data for SNP heritability and genetic correlation analysis. Bioinformatics. 2017;33:272–279.

[19] de Leeuw CA, Stringer S, Dekkers IA, Heskes T, Posthuma D. Conditional and interaction gene-set analysis reveals novel functional pathways for blood pressure. Nat. Commun. 2018;9:3768.

[20] GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. Nat. Genet. 2013;45:580–585.

[21] Koivula RW, Forgie IM, Kurbasic A, Viñuela A, Heggie A, Giordano GN, et al. Discovery of biomarkers for glycaemic deterioration before and after the onset of type 2 diabetes: an overview of the data from the epidemiological studies within the IMI DIRECT Consortium [Internet]. bioRxiv. 2018 [cited 2019 Feb 7];300244. Available from: https://www.biorxiv.org/content/10.1101/300244v1

[22] Dongiovanni P, Fracanzani AL, Fargion S, Valenti L. Iron in fatty liver and in the metabolic syndrome: a promising therapeutic target. J. Hepatol. 2011;55:920–932.

[23] Dongiovanni P, Lanti C, Gatti S, Rametta R, Recalcati S, Maggioni M, et al. High fat diet subverts hepatocellular iron uptake determining dysmetabolic iron overload. PLoS One. 2015;10:e0116855.

[24] Paisant A, d'Assignies G, Bannier E, Bardou-Jacquet E, Gandon Y. MRI for the measurement of liver iron content, and for the diagnosis and follow-up of iron overload disorders. Presse Med. 2017;46:e279–e287.

[25] Pierce BL, Burgess S. Efficient Design for Mendelian Randomization Studies: Subsample and 2-Sample Instrumental Variable Estimators. Am. J. Epidemiol. 2013;178:1177–1184.

[26] Bowden J, Del Greco M F, Minelli C, Davey Smith G, Sheehan NA, Thompson JR. Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic. Int. J. Epidemiol. 2016;45:1961–1974.

[27] Burgess S, Bowden J, Fall T, Ingelsson E, Thompson SG. Sensitivity Analyses for Robust Causal Inference from Mendelian Randomization Analyses with Multiple Genetic Variants. Epidemiology. 2017;28:30–42.

[28] Rees JMB, Wood AM, Burgess S. Extending the MR-Egger method for multivariable Mendelian randomization to correct for both measured and unmeasured pleiotropy. Stat. Med. [Internet]. 2017;Available from: http://doi.wiley.com/10.1002/sim.7492

[29] Canela-Xandri O, Rawlik K, Tenesa A. An atlas of genetic associations in UK Biobank. Nat. Genet. 2018;50:1593–1599.

[30] Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome. Elife [Internet]. 2018;7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5976434

[31] Nuttall KL, Palaty J, Lockitch G. Reference limits for copper and iron in liver biopsies. Ann.

Clin. Lab. Sci. 2003;33:443–450.

[32] Nikpay M, Goel A, Won H-H, Hall LM, Willenborg C, Kanoni S, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. Nat. Genet. 2015;47:1121–1130.

[33] Paternoster L, Standl M, Waage J, Baurecht H, Hotze M, Strachan DP, et al. Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. Nat. Genet. 2015;47:1449–1456.

[34] Lu Y, Day FR, Gustafsson S, Buchkovich ML, Na J, Bataille V, et al. New loci for body fat percentage reveal link between adiposity and cardiometabolic disease risk. Nat. Commun. 2016;7:10495.

[35] Wallace DF, Summerville L, Crampton EM, Frazer DM, Anderson GJ, Subramaniam VN. Combined deletion of Hfe and transferrin receptor 2 in mice leads to marked dysregulation of hepcidin and iron overload. Hepatology. 2009;50:1992–2000.

[36] Guo S, Casu C, Gardenghi S, Booten S, Aghajan M, Peralta R, et al. Reducing TMPRSS6 ameliorates hemochromatosis and β-thalassemia in mice. J. Clin. Invest. 2013;123:1531–1541.

[37] Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science. 2004;306:2090–2093.

[38] Nai A, Pagani A, Silvestri L, Campostrini N, Corbella M, Girelli D, et al. TMPRSS6 rs855791 modulates hepcidin transcription in vitro and serum hepcidin levels in normal individuals. Blood. 2011;118:4459–4462.

[39] Barton JC, Edwards CQ, Acton RT. HFE gene: Structure, function, mutations, and associated iron abnormalities. Gene. 2015;574:179–192.

[40] Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J. Clin. Invest. 2007;117:175–184.

[41] Orr JS, Kennedy A, Anderson-Baucum EK, Webb CD, Fordahl SC, Erikson KM, et al. Obesity alters adipose tissue macrophage iron content and tissue iron distribution. Diabetes. 2014;63:421–432.

[42] Khan FA, Fisher MA, Khakoo RA. Association of hemochromatosis with infectious diseases: expanding spectrum. Int. J. Infect. Dis. 2007;11:482–487.

[43] Fonseca-Nunes A, Jakszyn P, Agudo A. Iron and cancer risk--a systematic review and meta-analysis of the epidemiological evidence. Cancer Epidemiol. Biomarkers Prev. 2014;23:12–31.

[44] Lv Y-F, Chang X, Hua R-X, Yan G-N, Meng G, Liao X-Y, et al. The risk of new-onset cancer associated with HFE C282Y and H63D mutations: evidence from 87,028 participants. J. Cell. Mol. Med. 2016;20:1219–1233.

[45] Elliott LT, Sharp K, Alfaro-Almagro F, Shi S, Miller KL, Douaud G, et al. Genome-wide association studies of brain imaging phenotypes in UK Biobank. Nature. 2018;562:210–216.

[46] Brar S, Henderson D, Schenck J, Zimmerman EA. Iron accumulation in the substantia nigra of patients with Alzheimer disease and parkinsonism. Arch. Neurol. 2009;66:371–374.

[47] Burgetova A, Seidl Z, Krasensky J, Horakova D, Vaneckova M. Multiple sclerosis and the accumulation of iron in the Basal Ganglia: quantitative assessment of brain iron using MRI t(2) relaxometry. Eur. Neurol. 2010;63:136–143.

[48] The Lancet Gastroenterology Hepatology. Ironing out unmet need in genetic haemochromatosis. Lancet Gastroenterol Hepatol. 2019;4:1.

[49] Ellervik C, Tybjaerg-Hansen A, Appleyard M, Ibsen H, Nordestgaard BG. Haemochromatosis genotype and iron overload: association with hypertension and left ventricular hypertrophy. J. Intern. Med. 2010;268:252–264.

[50] Cai H. Hydrogen peroxide regulation of endothelial function: origins, mechanisms, and consequences. Cardiovasc. Res. 2005;68:26–36.

[51] Pilling LC, Tamosauskaite J, Jones G, Wood AR, Jones L, Kuo C-L, et al. Common conditions associated with hereditary haemochromatosis genetic variants: cohort study in UK Biobank. BMJ. 2019;364:k5222.

[52] Gill D, Del Greco M F, Walker AP, Srai SKS, Laffan MA, Minelli C. The Effect of Iron Status on Risk of Coronary Artery Disease: A Mendelian Randomization Study-Brief Report. Arterioscler. Thromb. Vasc. Biol. 2017;37:1788–1792.

[53] Fry A, Littlejohns TJ, Sudlow C, Doherty N, Adamska L, Sprosen T, et al. Comparison of Sociodemographic and Health-Related Characteristics of UK Biobank Participants With Those of the General Population. Am. J. Epidemiol. 2017;186:1026–1034.

Figure 1. Study design. GWAS on liver iron content was performed in UK Biobank (N = 8,289), and replicated in IMI DIRECT (N = 1,115).

Figure 2. Manhattan plot illustrating genetic variants (~30 million imputed SNPs) associated with liver iron in UK Biobank. The x-axis is the chromosomal position and y axis is -log(P) for the association with each variant. Black line indicates genome-wide significance level (5×10^{-8}).

Figure 3. Liver iron content per genotype group. X-axis are the 6 genotypes groups based on the number of C282Y and H63D they carry. Y-axis is the mean of liver iron (mg/g) per category. Error bars indicate 95% confidence intervals. Numbers in brackets are the number of individuals per genotype category.

Figure 4. The effect of 25 predominantly metabolic traits and diseases on liver iron content (standard deviation (SD)). The plot illustrates per allele effect of genetic variants associated with different metabolic traits. We have used alleles associated with higher adiposity, higher disease risk, higher glycaemic traits, adverse lipid levels, higher liver enzymes and adverse metabolic biomarkers profile. In comparison, the plot illustrates the effect of *HFE* C282Y, *HFE* H63D, *TMPRSS6* V736A on liver iron content. Please refer to **Supplementary Table 10** for the results of two-sample Mendelian randomisation analysis. The error bars indicate 95% confidence intervals.

Figure 5. Illustration of prioritised associations following phenome-wide association studies (PheWAS) of rs1800562, rs1799945 and rs855791 and significant traits from UK Biobank and publicly available summary statistics. Blue indicates a positive association and red an inverse association, following correction for multiple testing (False discovery rate < 5%). Continuous traits betas were scaled to per

SD where appropriate for better visualisation. Effect on disease risk is given in log(odds ratio).