Hepcidin-regulating iron metabolism genes and pancreatic ductal adenocarcinoma: a pathway analysis of genome-wide association studies

Sachelly Julián-Serrano,[1] Fangcheng Yuan,[1] William Wheeler,[2] Beben Benyamin,[3,4] Mitchell J Machiela,[1] Alan A Arslan,[5] Laura E Beane-Freeman,[1] Paige M Bracci,[6] Eric J Duell,[7,8,9] Mengmeng Du,[10] Steven Gallinger,[11] Graham G Giles,[12,13,14] Phyllis J Goodman,[15] Charles Kooperberg,[16] Loic Le Marchand,[17] Rachel E Neale,[18] Xiao-Ou Shu,[19] Stephen K Van Den Eeden,[20] Kala Visvanathan,[21] Wei Zheng,[19] Demetrius Albanes,[1] Gabriella Andreotti,[1] Eva Ardanaz,[22,23,24] Ana Babic,[25] Sonja I Berndt,[1] Lauren K Brais,[25] Paul Brennan,[26] Bas Bueno-de-Mesquita,[27] Julie E Buring,[28] Stephen J Chanock,[1] Erica J Childs,[29] Charles C Chung,[1] Eleonora Fabiánová,[30] Lenka Foretová,[31] Charles S Fuchs,[32] J Michael Gaziano,[33] Manuel Gentiluomo,[34,35] Edward L Giovannucci,[25] Michael G Goggins,[36] Thilo Hackert,[37] Patricia Hartge,[1] Manal M Hassan,[38] Ivana Holcátová,[39] Elizabeth A Holly,[6] Rayjean I Hung,[11] Vladimir Janout,[40] Robert C Kurtz,[41] I-Min Lee,[28,42] Núria Malats,[43] David McKean,[29] Roger L Milne,[12,13,14] Christina C Newton,[44] Ann L Oberg,[45] Sandra Perdomo,[26] Ulrike Peters,[16] Miquel Porta,[46] Nathaniel Rothman,[1] Matthias B Schulze,[47,48] Howard D Sesso,[28] Debra T Silverman,[1] Ian M Thompson,[49] Jean Wactawski-Wende,[50] Elisabete Weiderpass,[16] Nicolas Wenstzensen,[1] Emily White,[16] Lynne R Wilkens,[17] Herbert Yu,[17] Anne Zeleniuch-Jacquotte,[51] Jun Zhong,[1] Peter Kraft,[42,52] Dounghui Li,[53] Peter T Campbell,[44] Gloria M Petersen,[45] Brian M Wolpin,[25] Harvey A Risch,[54] Laufey T Amundadottir,[1] Alison P Klein,[29,36] Kai Yu,[1] and Rachael Z Stolzenberg-Solomon[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD, USA; [2]Information Management Services, Silver Spring, MD, USA; [3]Australian Centre for Precision Health, Allied Health and Human Performance, University of South Australia, Adelaide, Australia; [4]South Australian Health and Medical Research Institute, Adelaide, Australia; [5]Department of Obstetrics and Gynecology, New York University School of Medicine, New York, NY, USA; [6]Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, USA; [7]Unit of Biomarkers and Susceptibility, Oncology Data Analytics Program, Catalan Institute of Oncology, L'Hospitalet de Llobregat, Barcelona, Spain; [8]Colorectal Cancer Group, ONCOBELL Program, Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain; [9]Consortium for Biomedical Research in Epidemiology and Public Health (CIBERESP), Madrid, Spain; [10]Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, USA; [11]Lunenfeld–Tanenbaum Research Institute, Sinai Health System, Toronto, Canada; [12]Cancer Epidemiology Division, Cancer Council Victoria, Melbourne, Australia; [13]Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Australia; [14]Precision Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton, Australia; [15]SWOG Statistical Center, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [16]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [17]Department of Epidemiology, University of Hawaii Cancer Center, Honolulu, HI, USA; [18]Population Health Department, QIMR Berghofer Medical Research Institute, Brisbane, Australia; [19]Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt–Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN, USA; [20]Division of Research, Kaiser Permanente Northern California, Oakland, CA, USA; [21]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; [22]Navarra Public Health Institute, Pamplona, Spain; [23]IdiSNA, Navarra Institute for Health Research, Pamplona, Spain; [24]CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain; [25]Department of Medical Oncology, Dana–Farber Cancer Institute, Boston, MA, USA; [26]International Agency for Research on Cancer (IARC), Lyon, France; [27]Department for Determinants of Chronic Diseases, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands; [28]Division of Preventive Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA; [29]Department of Oncology, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA; [30]Specialized Institute of Hygiene and Epidemiology, Banska Bystrica, Slovakia; [31]Department of Cancer Epidemiology and Genetics, Masaryk Memorial Cancer Institute, Brno, Czech Republic; [32]Yale Cancer Center and Smilow Cancer Hospital, New Haven, CT, USA; [33]Division of Aging, Brigham and Women's Hospital, Boston, MA, USA; [34]Department of Biology, University of Pisa, Italy; [35]Genomic Epidemiology Group, German Cancer Research Center, (DKFZ), Heidelberg, Germany; [36]Department of Pathology, Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins School of Medicine, Baltimore, MD, USA; [37]Department of General, Visceral and Transplantation Surgery, Heidelberg University Hospital, Heidelberg, Germany; [38]Department of Epidemiology, University of Texas MD Anderson Cancer Center, Houston, TX, USA; [39]Institute of Public Health and Preventive Medicine, Second Faculty of Medicine, Charles University, Prague, Czech Republic; [40]Faculty of Health Sciences, University of Olomouc, Olomouc, Czech Republic; [41]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA; [42]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA; [43]Genetic and Molecular Epidemiology Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain; [44]Department of Population Science, American Cancer Society, Atlanta, GA, USA; [45]Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN, USA; [46]Hospital del Mar Institute of Medical Research (IMIM), Universitat Autònoma de Barcelona, Barcelona, Spain; [47]Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany; [48]Institute of Nutritional Science, University of Potsdam, Nuthetal, Germany; [49]CHRISTUS Santa Rosa Hospital–Medical Center, San Antonio, TX, USA; [50]Department of Epidemiology and Environmental Health, University at Buffalo, Buffalo, NY, USA; [51]Department of Population Health and Perlmutter Cancer

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Center, New York University School of Medicine, New York, NY, USA; [52]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA; [53]Department of Gastrointestinal Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; and [54]Department of Chronic Disease Epidemiology, Yale School of Public Health, New Haven, CT, USA

ABSTRACT

Background: Epidemiological studies have suggested positive associations for iron and red meat intake with risk of pancreatic ductal adenocarcinoma (PDAC). Inherited pathogenic variants in genes involved in the hepcidin-regulating iron metabolism pathway are known to cause iron overload and hemochromatosis.

Objectives: The objective of this study was to determine whether common genetic variation in the hepcidin-regulating iron metabolism pathway is associated with PDAC.

Methods: We conducted a pathway analysis of the hepcidin-regulating genes using single nucleotide polymorphism (SNP) summary statistics generated from 4 genome-wide association studies in 2 large consortium studies using the summary data-based adaptive rank truncated product method. Our population consisted of 9253 PDAC cases and 12,525 controls of European descent. Our analysis included 11 hepcidin-regulating genes [bone morphogenetic protein 2 (*BMP2*), bone morphogenetic protein 6 (*BMP6*), ferritin heavy chain 1 (*FTH1*), ferritin light chain (*FTL*), hepcidin (*HAMP*), homeostatic iron regulator (*HFE*), hemojuvelin (*HJV*), nuclear factor erythroid 2-related factor 2 (*NRF2*), ferroportin 1 (*SLC40A1*), transferrin receptor 1 (*TFR1*), and transferrin receptor 2 (*TFR2*)] and their surrounding genomic regions (±20 kb) for a total of 412 SNPs.

Results: The hepcidin-regulating gene pathway was significantly associated with PDAC ($P = 0.002$), with the *HJV*, *TFR2*, *TFR1*, *BMP6*, and *HAMP* genes contributing the most to the association.

Conclusions: Our results support that genetic susceptibility related to the hepcidin-regulating gene pathway is associated with PDAC risk and suggest a potential role of iron metabolism in pancreatic carcinogenesis. Further studies are needed to evaluate effect modification by intake of iron-rich foods on this association. *Am J Clin Nutr* 2021;00:1–10.

Keywords: hepcidin, iron metabolism pathway, pancreatic cancer, genetic susceptibility, epidemiology

Introduction

In the United States, pancreatic cancer is the third leading cause of cancer mortality, and its incidence is increasing (1). Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer (2). Known risk factors for PDAC include cigarette smoking, excess weight, type 2 diabetes, and heavy alcohol consumption. Genetic susceptibility also plays a role, with an estimated heritability of up to 21% (3).

Epidemiological studies have shown associations between greater consumption of red meat (4–7) and heme iron (8) and increased PDAC risk. Higher serum iron has also been found to be associated with PDAC, although not consistently (9–11). Experimental studies of iron overload support the hypothesis that iron accumulates in pancreatic islets, resulting in reduced insulin secretion, increased pancreatic β-cell death, and decreased pancreatic β-cell function (12, 13) that contributes to diabetes, a known risk factor for PDAC. Free iron catalyzes

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the generation of reactive oxygen species (ROS), potentially promoting oxidative stress and inflammation (12).

Hepcidin, a 25–amino acid peptide hormone, maintains iron homeostasis and tightly regulates circulating iron by binding to and degrading iron's receptor protein, ferroportin (14). This process inhibits iron absorption in the duodenum, release of recycled iron from macrophages, and release of stored iron from hepatocytes (15). Although hepcidin is primarily synthesized and secreted by the liver, it can also be produced by macrophages, pancreatic islets, and adipose tissue (15). Hepcidin is produced when iron is abundant and in response to inflammation as part of the acute phase response and erythropoiesis (16). The genes involved in hepcidin regulation are highly conserved in vertebrates (17). Hereditary hemochromatosis is caused by mutations in 5 genes, namely hepcidin (*HAMP*), homeostatic iron regulator (*HFE*), hemojuvelin (*HJV*; also known as *HFE2*), solute carrier family 40 (iron-regulated transporter) member 1 or ferroportin 1 (*SLC40A1*), and transferrin receptor 2 (*TFR2*). Mutations in these genes result in insufficient production of hepcidin and promote excess iron absorption from the diet and accumulation of iron in tissues and organs, most notably the liver, pancreas, and heart (18). Hemochromatosis is associated with pancreatogenic diabetes (19) and hepatocellular carcinoma (18).

The goal of the present analysis was to determine whether the hepcidin-regulating iron metabolism pathway as characterized by common variants in hepcidin-regulating genes is associated with risk of PDAC. For this analysis, we focus on genes involved in iron sensing and regulation of dietary iron absorption. Given the role of hepcidin-regulating genes in hemochromatosis and pancreatogenic diabetes, we hypothesize that the hepcidin-regulating gene pathway is associated with PDAC risk.

Methods

The Pancreatic Cancer Cohort Consortium and the Pancreatic Cancer Case–Control Consortium

Our analysis included 9,253 primary PDAC cases (ICD-O-3 code C250–C259) and 12,525 controls that were part of 4 genome-wide association studies (GWAS) conducted in the Pancreatic Cancer Cohort (PanScan) I–III Consortium and the Pancreatic Cancer Case–Control (PanC4) Consortium (20–24) (**Supplemental Figure 1**). Details of the studies have been previously described (20–23). We only included participants of European ancestry based on the genomic data (20–23) to avoid confounding by population stratification. Pancreatic cancer cases with non-PDAC subtypes (histology types 8150, 8151, 8153, 8155, and 8240) were excluded because their etiologies are thought to be different. The 3 PanScan GWAS included participants from 16 cohorts from the National Cancer Institute (NCI) Cohort Consortium, 9 case–control studies, and 1 case series (Gastrointestinal Cancer Clinic of Dana–Farber Cancer Institute) (22). PanC4 included 9 case–control studies (23). Within the individual studies, controls for PanScan I and II and PanC4 were matched to cases on age, sex, race, area of residence (case–control studies) and/or smoking (Health Professionals

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Supplemental Figures 1 and 2 and Supplemental Tables 1–6 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

Address correspondence to RZS-S (e-mail: rs221z@nih.gov).

Abbreviations used: *BMP*, bone morphogenetic protein; eQTL, expression quantitative trait loci; *FTH1*, ferritin heavy chain 1; *FTL*, ferritin light chain; GTEx, Genotype-Tissue Expression; GWAS, genome-wide association study; *HAMP*, hepcidin; *HFE*, homeostatic iron regulator; *HJV*, hemojuvelin; LD, linkage disequilibrium; NCI, National Cancer Institute; *NRF2*, nuclear factor erythroid 2-related factor 2; PanC4, Pancreatic Cancer Case–Control Consortium; PanScan, Pancreatic Cancer Cohort Consortium; PDAC, pancreatic ductal adenocarcinoma; RDW, red blood cell distribution width; ROS, reactive oxygen species; sARTP, summary data-based adaptive rank truncated product; *SLC40A1*, ferroportin 1; SNP, single nucleotide polymorphism; *TFR*, transferrin receptor.

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Follow-Up Study, Physicians' Health Study, Nurses' Health Study, and Women's Health Study only), and incidence density sampled within each respective cohort study (20–23). PanScan III used previously genotyped controls, mostly from cohort studies. Each participating study obtained written informed consent from their participants and approval from their local Institutional Review Board. The NCI's Special Studies Institutional Review Board approved the consortia study.

The genotyping methods, quality control, and imputation for the originating GWAS have been previously described (20–24). The genotyping for PanScan studies was performed at the NCI's Division of Cancer Epidemiology and Genetics' Cancer Genomics Research Laboratory using Illumina HumanHap series arrays [Illumina HumanHap550 Infinium II array (20) and Human 610-Quad array (21) for PanScan I and II, respectively; Illumina Omni series arrays (OmniExpress, Omni1M, Omni2.5M, and Omni5M) for PanScan III (22)]. The PanC4 study was genotyped on the Illumina HumanOmniExpressExome-8v1 array at the Johns Hopkins Center for Inherited Disease Research (23). Genotype imputation across the 4 study phases was based on the 1000 Genomes Project (phase 3, v1) reference data set using IMPUTE2 (http://mathgen.stats.ox.ac.uk/impute/impute_v2.html) (24, 25). For quality control, single nucleotide polymorphisms (SNPs) with low-quality imputation score (IMPUTE2 INFO score < 0.3) were excluded (24).

Selection of hepcidin regulatory genes and pathway analysis for pancreatic cancer

Regulation of systemic iron occurs through the hepcidin–ferroportin axis (26–28). We selected genes involved in hepcidin regulation through iron-sensing signals (28). These include bone morphogenetic protein 2 (*BMP2*), bone morphogenetic protein 6 (*BMP6*), *HAMP*, *HFE*, *HJV* (also known as *HFE2*), nuclear factor erythroid 2-related factor 2 (*NRF2*), transferrin receptor 1 (*TFR1*), and *TFR2*. We also included 3 genes in the hepcidin–ferroportin complex, namely *SLC40A1*, ferritin heavy chain 1 (*FTH1*), and ferritin light chain (*FTL*). For the gene-based analyses, we mapped SNPs within a genomic region 20 kb upstream and 20 kb downstream of each of the 11 genes. In total, our analysis included 412 SNPs. We excluded SNPs with minor allele frequency <5% and applied linkage disequilibrium (LD) filtering to highly correlated SNP pairs ($r^2 > 0.81$).

Due to the large overlap of variants on the arrays (Illumina HumanHap550 Infinium II, Human 610-Quad) used for PanScan I and II, these studies were combined and jointly analyzed, whereas PanScan III (OmniExpress, Omni1M, Omni2.5M, and Omni5M) and PanC4 (Illumina HumanOmniExpressExome-8v1) were each analyzed separately. The SNP association analysis was conducted using logistic regression with the SNPs coded as the number of effect alleles in the observed genotype or the expected number of effect alleles for imputed genotype [Prob(SNP = effect allele | reference allele) + 2Prob(SNP = effect allele | effect allele)], adjusted for age (10-y categories: (≤50, 51–60, 61–70, 71–80, and ≥81 y)], sex, top eigenvectors for each study phase, study (PanScan), and geographic region (PanScan). We conducted a meta-analysis combining SNP-level summary statistics from the 4 GWAS using an inverse-variance fixed-effects model.

We then performed a gene- and pathway-based analysis using the summary data-based adaptive rank truncated product (sARTP) method, which combines the SNP-level associations across SNPs in a gene or a pathway (29). Signals from ≤5 of the most PDAC-associated SNPs in a gene region were accumulated. The sARTP adjusts for the number of SNPs in a gene and the number of genes in a pathway through a resampling procedure that controls for false positives. The *P* values of gene- and pathway-level associations were estimated from the resampled null distribution generated from 1 million permutations. We also performed analyses by subgroup, including sex, age at diagnosis with PDAC (<60 vs. ≥ 60 y), and BMI (in kg/m^2; <25, 25 to <30, ≥30, and missing). The statistical analyses were performed using the R programing language (version 3.6.3; R Foundation for Statistical Computing). We considered the pathway-level *P* value < 0.05 to be statistically significant. All tests were 2-sided.

Expression quantitative trait loci analysis and functional annotation

We conducted an exploratory analysis of expression quantitative trait loci (eQTL) data to assess the *cis* effect of sARTP-selected SNPs in pancreas ($n = 305$) and other tissues using data from the NIH Genotype-Tissue Expression project (GTEx) v8 (https://www.gtexportal.org/home) and reported expression significant at an false discover rate <0.05 (30). We also examined the regulatory potential of these SNPs (and SNPs in LD) using data and information from HaploReg (31) and RegulomeDB v1.1 (32). If an SNP in our data set was not genotyped or imputed in HaploReg or RegulomeDB v1.1, we selected an alternative SNP in high LD ($r^2 \geq 0.90$) using LDlink (https://ldlink.nci.nih.gov) (33).

Exploratory analysis with iron and hematologic traits

We also conducted exploratory analyses examining the association between the sARTP-selected SNPs with biomarkers of iron status (serum iron, transferrin, transferrin saturation, and ferritin) and hematologic traits including hemoglobin, hematocrit, RBC count, and RBC distribution width (RDW) using summary statistics from 2 published GWAS (34, 35). We explored the association between the sARTP-selected SNPs and hematologic traits because the majority of iron in the body is contained in the hemoglobin. The iron status biomarkers GWAS consisted of 23,986 individuals of European ancestry (34). The hematologic traits GWAS was from the UK Biobank cohort Gene ATLAS of 452,264 British individuals of European ancestry (35). For SNPs in our data that were not genotyped or imputed in the other 2 data sets (i.e., genotyped on different platforms), we selected an alternative index SNP in high LD ($r^2 \geq 0.90$) using LDlink (33). We then standardized the β coefficients to create a heatmap for comparing the SNP associations across the iron status biomarkers and hematologic traits. To standardized β values in both data sets, we calculated *z* scores, dividing the β by the SE. Because we did not have the SE for the UK Biobank GWAS data on blood traits but had the *P* values, the *z* score was obtained using the inverse normal distribution function applied to each *P* value divided by 2. We also conducted exploratory analyses of the hepcidin regulatory gene pathway in relation to serum iron, transferrin saturation, ferritin, and transferrin, respectively, using

the SNP-level summary statistics from the Genetics of Iron Status Consortium (29, 34) and sARTP, applying similar methods as described previously. In total, these analyses included up to 180 SNPs.

Results

Baseline characteristics of the PanScan and PanC4 studies are shown in **Table 1**. Most PDAC cases were diagnosed at age >60 y. The sex and age distributions of the cases compared to controls were similar within PanScan I and II and PanC4. Compared with the cases, the PanScan III controls had a higher proportion of men and participants <70 y old when selected because PanScan III used previously genotyped controls. Overall, cases were more often men (54.1%), with PanC4 having the highest proportion (57.5%) and the PanScan III having the lowest proportion (49.4%) of men.

The hepcidin-regulating iron metabolism pathway was significantly associated with PDAC ($P = 0.0028$) (**Figure 1**). The genes (and corresponding SNPs; **Table 2**) selected by sARTP that contributed the most to the overall association were *HJV* (rs6424377, rs10910813, rs2027387, rs10910810, rs10910809), *TFR2* (rs62482223, rs56328569), *TFR1* (rs4927870, rs41297523, rs13093426, rs41299394, rs12487702), *BMP6* (rs61668994), and *HAMP* (rs10419959, rs12981457, rs10421768, rs10424619, rs2284147). In subgroup analyses (**Supplemental Table 1**), the pathway association tended to be stronger for women (pathway P value $= 0.005$; *HJV*), participants aged ≥60 y (pathway P value $= 0.007$; *HJV*, *BMP6*, *TFR1*, *FTL*), and participants who were obese (BMI >30; pathway P value $= 0.01$; *TFR2*, *HJV*, *FTH1*, *TFR1*), whereas their respective other strata showed no significant pathway associations (pathway P value > 0.05). The pathway was not significant in analyses stratified by median total meat (531 cases, 3,747 controls) or dietary iron intake (402 cases, 3,412 controls) (Supplemental Table 1).

In the exploratory analyses, the hepcidin-regulating iron metabolism pathway was significantly associated with all the iron status biomarkers (**Supplemental Table 2**; pathway P values $< 1.50 \times 10^{-7}$). All of the PDAC SNPs selected by sARTP for each gene were associated with either biomarkers of iron status or hematologic traits (**Supplemental Figure 2**, **Supplemental Tables 3** and **4**). The *HJV* variants were only associated with hematologic traits (hemoglobin, hematocrit, RBC, and RDW; P values < 0.05 and $>5.0 \times 10^{-6}$). The *TFR2* SNPs were positively associated with all the hematologic traits (P values $< 5.0 \times 10^{-10}$) and inversely associated with serum iron and transferrin saturation (P values < 0.05). The *TFR1* SNPs were associated inversely with transferrin saturation and positively with ferritin or transferrin (P values < 0.05 and $>5.0 \times 10^{-4}$). The *TFR1* SNPs were also associated with RBC count and RDW (P values < 0.05 and $>5.0 \times 10^{-11}$). The sARTP-selected *BMP6* SNP was associated with RDW (P value < 0.005 and $\geq 5.0 \times 10^{-5}$). The *HAMP* SNPs were very significantly associated with RBC and RDW (P values < 0.001 and $>5.0 \times 10^{-16}$) and less strongly associated with hemoglobin and hematocrit (P values < 0.05 and ≥ 0.005).

We examined eQTL results from GTEx (false discovery rate < 0.05) for the most significant sARTP-selected SNPs in pancreas tissue (**Table 3**) and other tissues (**Supplemental Table**

TABLE 1 Descriptive characteristics of participants by study phase from the PanScan and PanC4 studies[1]

Characteristic	Study phase				Total
	PanScan I case/control 1746/1812	PanScan II case/control 1768/1841	PanScan III case/control 1576/5080	PanC4 case/control 4163/3792	Combined case/control 9253/12,525
Case diagnosis age >60 y, *n* (%)	1375 (78.8)	1234 (69.8)	1192 (75.6)	2868 (68.9)	6669 (72.1)
Age,[2] *n* (%)					
<50 y	136 (7.8)/80 (4.4)	132 (7.5)/150 (8.1)	176 (11.2)/522 (10.3)	390 (9.4)/419 (11.0)	834 (9.0)/1171 (9.3)
50–59 y	235 (13.5)/264 (14.6)	402 (22.7)/394 (21.4)	208 (13.2)/1326 (26.1)	905 (21.7)/950 (25.1)	1750 (18.9)/2934 (23.4)
60–69 y	617 (35.3)/690 (38.1)	619 (35.0)/605 (32.9)	433 (27.5)/2474 (48.7)	1474 (35.4)/1266 (33.4)	3143 (34.0)/5035 (40.2)
≥70 y	758 (43.4)/778 (42.9)	615 (34.8)/692 (37.6)	759 (48.2)/758 (14.9)	1394 (33.5)/1157 (30.5)	3526 (38.1)/3385 (27.0)
Sex, *n* (%)					
Men	892 (51.1)/925 (51.0)	945 (53.5)/965 (52.4)	778 (49.4)/3795 (74.7)	2395 (57.5)/2106 (55.5)	5010 (54.1)/7791 (62.2)
Women	854 (48.9)/887 (49.0)	823 (46.5)/876 (47.6)	798 (50.6)/1285 (25.3)	1768 (42.5)/1686 (44.5)	4243 (45.9)/4734 (37.8)

[1] PanC4, Pancreatic Cancer Case–Control Consortium; PanScan, Pancreatic Cancer Cohort Consortium.
[2] Age at pancreatic cancer diagnosis or age when selected to be a control. PanScan III used previously genotyped controls.

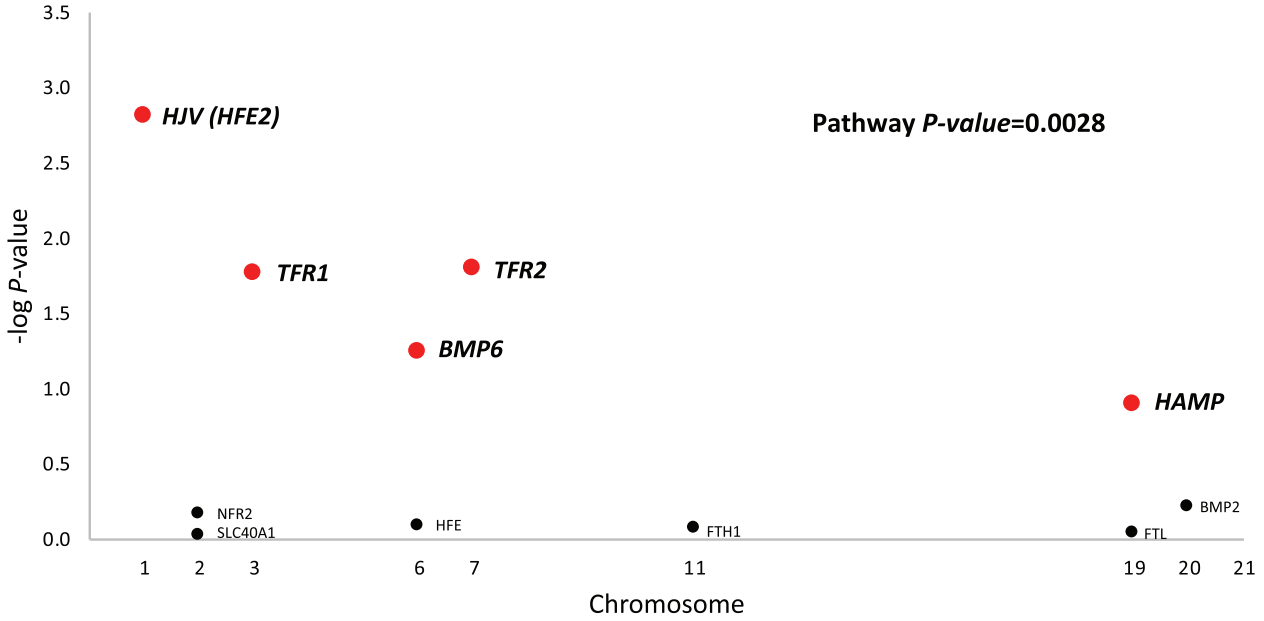

FIGURE 1 Genes in the hepcidin-regulating iron metabolism pathway associated with PDAC. The red circles are genes selected by sARTP as contributing the most to the pathway association, and black circles are genes not selected by sARTP. *HJV*, *TFR2*, *TFR1*, *BMP6*, and *HAMP* contributed to the pathway. The analysis used meta-analysis summary statistic SNP data from 4 genome-wide association studies (9,253 PDAC cases and 12,525 controls), includes 11 genes and 412 SNPs and is adjusted for age, sex, and the top eigenvectors for the PanScan studies and age, sex, and the top eigenvectors for the PanC4 studies. All statistical tests are 2-sided. *BMP2*, bone morphogenetic protein 2; *BMP6*, bone morphogenetic protein 6; *FTH1*, ferritin heavy chain 1; *HAMP*, hepcidin; *HFE*, homeostatic iron regulator; *HJV*, hemojuvelin; *NRF2*, nuclear factor erythroid 2-related factor 2; PanC4, Pancreatic Cancer Case–Control Consortium; PanScan, Pancreatic Cancer Cohort Consortium; PDAC, pancreatic ductal adenocarcinoma; sARTP, summary data-based adaptive rank truncated product; *SLC40A1*, ferroportin 1; SNP, single nucleotide polymorphism; *TFR1*, transferrin receptor 1; *TFR2*, transferrin receptor 2.

5), as well as exploratory functional annotation (**Supplemental Table 6**). Alleles in 2 correlated SNPs selected for *HJV* (r^2 = 0.70) rs6424377-A compared with the G allele and rs10910809-G compared with the A allele were associated with increased Neuroblastoma breakpoint family member 10 (*NBPF10*; *P* values $< 3.0 \times 10^{-8}$) expression in normal pancreas tissue, as well as whole blood, esophagus–mucosa, esophageal–muscularis, esophagus–gastroesophageal junction, stomach, colon–sigmoid, colon–traverse, artery–aorta, artery–tibial, or cell-cultured fibroblasts (*P* values $< 2.0 \times 10^{-6}$). The T compared to the C allele of rs10910810 selected for *HJV* was associated with increased expression of long intergenic nonprotein coding RNA 1719 (*LINC01719*; *P* value $= 5.00 \times 10^{-5}$) expression in the normal pancreas tissue and decreased *NBPF10* expression in the blood (*P* value $= 1.6 \times 10^{-8}$). Alleles in 4 correlated SNPs selected for *HAMP*, rs10419959-A, rs12981457-T, rs10421768-G, and rs10424619-T were associated with lower upstream stimulatory factor 2 (*USF2*) expression in normal pancreas tissue (*P* values $< 1.0 \times 10^{-5}$) and esophagus–muscularis (*P* values $< 5.0 \times 10^{-6}$).

Discussion

We observed a significant association between the combined effects of common variants in the hepcidin-regulating iron metabolism gene pathway and PDAC. The signals contributing the most to the association were from the *HJV*, *TFR2*, *TFR1*, *BMP6*, and *HAMP* genes. Hepcidin is encoded by the *HAMP* gene and is the primary regulator of iron. When iron status is high, it triggers lysosomal degradation of ferroportin encoded by *SLC40A1* in the basolateral membrane of enterocytes and plasma membrane of macrophages (27). *HAMP* expression is regulated by the liver, sensing intracellular and extracellular iron (36). Hepatocyte *TFR1*, *TFR2*, and *HFE* sense extracellular iron, specifically high circulating concentrations of transferrin-bound iron or transferrin saturation, and signal increased *HAMP* transcription and hepcidin expression (36). Complementary to this iron-sensing mechanism, increased cellular iron stores also induce the production of *BMP6*, which acts in a paracrine manner to bind to heterodimeric BMP receptors and its coreceptor *HJV* activating the *SMAD* pathway, which stimulates *HAMP* transcription and hepcidin expression (27). Chronically low production of hepcidin can lead to increased blood iron concentrations, iron overload, and iron accumulation in the pancreas (27). Pathogenic variants in *HFE* [C282Y and p.His63Asp (H63D)] cause type 1 hemochromatosis, the most prevalent form of hemochromatosis. Although the *HFE*–H63D polymorphism (but not *HFE*–C282Y) was significantly associated with increased PDAC risk in an Asian population (37, 38), the *HFE* gene did not contribute to our pathway association. Among the 5 genes most contributing to our association, 4 (*HJV*, *HAMP*, *TFR2*, *BMP6*) are known to play a role in non-*HFE* hemochromatosis. Mutations in *HJV* and *HAMP* contribute to juvenile or type 2 hemochromatosis, and mutations in *TFR2* contribute to young adult type 3 hemochromatosis. *BMP6* mutations may contribute to late-onset moderate iron overload and hereditary hemochromatosis (18).

TABLE 2 Top SNPs (P values < 0.05) for most significant hepcidin-regulating genes in association with PDAC in the PanScan and PanC4 studies[1]

Gene in pathway	Location	Selected SNPs	Chr	Position[2]	r^2 [3]	EA/RA[4]	EAF	Allelic OR (95% CI)[5]	SNP P value[6]
HJV (*HFE2*)	1q21.1	rs6424377	1	145428400	Ref	A/G	0.41	0.93 (0.89, 0.97)	6.14×10^{-4}
		rs10910813	1	145426341	0.82	C/T	0.36	0.94 (0.90, 0.98)	2.82×10^{-3}
		rs2027387	1	145402014	0.57	G/C	0.47	1.06 (1.02, 1.11)	3.24×10^{-3}
		rs10910810	1	145399229	0.58	T/C	0.47	1.06 (1.02, 1.11)	3.48×10^{-3}
		rs10910809	1	145395618	0.70	G/A	0.37	0.94 (0.91, 0.99)	9.35×10^{-3}
TFR2	7q22.1	rs62482223	7	100198878	Ref	A/C	0.22	0.92 (0.88, 0.97)	1.27×10^{-3}
		rs56328569	7	100202219	0.97	G/C	0.23	0.92 (0.88, 0.97)	2.00×10^{-3}
TFR1	3q29	rs4927870	3	195820576	Ref	A/C	0.12	1.11 (1.04, 1.18)	1.51×10^{-3}
		rs41297523	3	195784648	0.64	C/T	0.08	1.13 (1.05, 1.22)	1.95×10^{-3}
		rs13093426	3	195828806	0.66	T/C	0.08	1.13 (1.05, 1.22)	2.02×10^{-3}
		rs41299394	3	195775824	0.62	T/C	0.07	1.13 (1.05, 1.23)	2.18×10^{-3}
		rs12487702	3	195775991	0.62	A/G	0.08	1.13 (1.04, 1.22)	2.51×10^{-3}
BMP6	6p24.3	rs61668994	6	7733746		T/C	0.14	1.11 (1.05, 1.19)	4.95×10^{-4}
HAMP	19q13.12	rs10419959	19	35764705	Ref	A/G	0.21	1.07 (1.02, 1.13)	5.53×10^{-3}
		rs12981457	19	35766595	0.68	T/C	0.17	1.07 (1.01, 1.13)	1.77×10^{-2}
		rs10421768	19	35772899	0.89	G/A	0.23	1.06 (1.01, 1.11)	1.87×10^{-2}
		rs10424619	19	35768237	0.93	T/A	0.22	1.06 (1.01, 1.11)	2.30×10^{-2}
		rs2284147	19	35765041	0.29	A/G	0.48	1.05 (1.00, 1.09)	3.34×10^{-2}

[1]Top SNPs (up to 5 for each gene) from each significant gene in the sARTP gene pathway analysis for hepcidin-regulating genes-PDAC association derived from 9,253 PDAC cases and 12,525 controls. A, adenine; *BMP6*, bone morphogenetic protein 6; C, cytosine; Chr, chromosome; EA, effect allele; EAF, effect allele frequency; G, guanine; *HAMP*, hepcidin; *HFE*, homeostatic iron regulator; *HJV*, hemojuvelin; PanC4, Pancreatic Cancer Case–Control Consortium; PanScan, Pancreatic Cancer Cohort Consortium; PDAC, pancreatic ductal adenocarcinoma; RA, referent allele; sARTP, summary data-based adaptive rank truncated product; SNP, single nucleotide polymorphism; T, thymine; *TFR1*, transferrin receptor 1; *TFR2*, transferrin receptor 2.

[2]Base pair coordinate hg19.

[3]Linkage disequilibrium (r^2) values are indicated between SNPs selected by sARTP and are derived from LDlink (https://ldlink.nci.nih.gov/) in European population data.

[4]Effect allele is defined as the minor allele.

[5]OR from unconditional logistic regression adjusted for study, geographical region, age, sex, and to principal components of population substructure in each of the study phases.

[6]Most significant SNP P value based on permutation.

Experimental and PDAC patient survival studies of hepcidin support our findings and may offer insight into biologic mechanisms. In a study of pancreatic cancer tissues from 92 patients who received curative resection, higher hepcidin tissue expression was associated with significantly poorer overall survival and was correlated with more advanced PDAC stage and vascular invasion (39). Local upregulation expression of hepcidin has also been reported in other tumor tissues (40–45). The increased hepcidin expression by cancer cells is hypothesized to be an adaptation that affects iron export and contributes to iron retention in tumor cells, helping them survive and proliferate (45). Furthermore, *TFR1* was highly expressed in PDAC cells and

TABLE 3 eQTL for hepcidin iron-regulating gene pathway SNPs in normal pancreas from the GTEx project[1]

Pathway gene	Location	r^2 [2]	SNP-EA	GTEx pancreas (n = 305) eQTL gene[3]	P value[4]	β (SE)[5]
HJV	1q21.1	Ref	rs6424377-A	*NBPF10*	2.2×10^{-8}	0.38 (0.06)
				LINC01719	6.7×10^{-6}	0.34 (0.07)
	1q21.1	0.58	rs10910810-T	*LINC01719*	5.0×10^{-5}	0.31 (0.07)
	1q21.1	0.70	rs10910809-G	*NBPF10*	2.9×10^{-8}	0.34 (0.07)
HAMP	19q13.12	Ref	rs10419959-A	*USF2*	1.0×10^{-5}	-0.21 (0.05)
	19q13.12	0.68	rs12981457-T	*USF2*	1.0×10^{-6}	-0.26 (0.05)
	19q13.12	0.89	rs10421768-G	*USF2*	8.3×10^{-6}	-0.20 (0.04)
	19q13.12	0.93	rs10424619-T	*USF2*	1.1×10^{-5}	-0.21 (0.05)

[1]Significant SNPs associated with pancreatic ductal adenocarcinoma in the PanScan and PanC4 studies with expression in pancreatic tissue. EA, effect allele; eQTL, expression quantitative trait loci; GTEx, Genotype-Tissue Expression; *HAMP*, hepcidin; *HJV*, hemojuvelin; PanC4, Pancreatic Cancer Case–Control Consortium; PanScan, Pancreatic Cancer Cohort Consortium; Ref, reference; SNP, single nucleotide polymorphism.

[2]Linkage disequilibrium (r^2) values are derived from LDlink (https://ldlink.nci.nih.gov/) in European population data.

[3]eQTL gene is defined as the gene in close proximity to the SNP.

[4]P value for the eQTL in pancreas has a false discovery rate <0.05.

[5]Effect sizes (β) and SE are computed as the effect of the effect allele relative to the reference allele.

was necessary for pancreatic cell proliferation and tumorigenesis through mitochondrial respiration and ROS formation (46). In a study that included tissues from 96 PDAC patients, iron content was higher in tumor tissue compared with adjacent normal tissue, and patients with high tumor *TFR1* expression had significantly worse overall and relapse-free survival compared with those with negative/low/medium expression (47). More research is needed to understand the role of the other genes selected in our pathway analysis in PDAC.

In our exploratory analyses, the hepcidin-regulating iron metabolism gene pathway overall was associated with all the iron status biomarkers, and the sARTP-selected SNPs for PDAC were associated with the iron status biomarkers and/or hematology traits. The sARTP-selected SNPs for *TFR1* were positively associated with PDAC (Table 2) and with ferritin, suggesting higher iron stores and greater PDAC risk (Supplemental Figure 2). Alleles in the sARTP-selected SNPs for *TFR2* (rs62482223-C, rs56328569-C) were inversely associated with PDAC and also inversely associated with serum iron, transferrin saturation, consistent with lower iron status contributing to reduced PDAC risk. The sARTP-selected *HAMP* SNP rs10421768 (G compared with the C allele) associated with increased PDAC in our study is in the 5′ flanking region of the *HAMP* gene encoding hepcidin and has been associated with higher liver (48) and cardiac tissue (49) and higher ferritin concentrations (48) in β-thalassemia patients. One study that included only men to avoid effects of physiologically lower serum ferritin levels in women due to menstruation reported rs10421768-G was associated with lower serum ferritin concentrations (50). Our analysis of participants in the Genetics of Iron Status Consortium and another study of 244 healthy individuals (51) did not demonstrate associations between rs10421768-G and serum iron, ferritin, or transferrin concentrations; however, neither study was able to stratify associations by sex. Although iron plays a key role in hematologic traits and some associations between the sARTP-selected SNPs with the hematologic traits were identified, it is unclear how this may be related to PDAC and thus requires further investigation.

Some sARTP-selected SNPs located in *HJV* and *HAMP*act as eQTLs in normal pancreas tissue, adding to the biologic plausibility of our findings. Alleles in 4 correlated sARTP-selected SNPs (rs10419959-A, rs12981457-T, rs10421768-G, rs10424619-T) in *HAMP* were associated with expression of *UFS2* in normal pancreas tissue. *USF2*plays a role in hepcidin regulation (52). Compared with wild type, *Usf2*$^{-/-}$ knockout mice had progressive iron accumulation up to 20-fold higher in the pancreas and 10-fold higher in the liver between 60 and 100 d after birth (52) and without hepcidin expression in the liver (51). We also observed eQTL signals in *NBPF10* for 2 correlated sARTP-selected *HJV* SNPs (rs6424377, rs10910810). Although *NBPF10*'s function is unknown, it is located in close proximity to *HJV*. Rare pathogenic mutations in both *HJV* and *HAMP* are known to play a role in juvenile hemochromatosis.

The strengths of our study include the large numbers of PDAC cases and controls and the statistical pathway approach using GWAS summary statistics, which allows for detection of the accumulative effect of multiple PDAC-associated variants within genes and surroundings regions in the hepcidin-regulating iron metabolism pathway. Our approach provides the opportunity to identify susceptibility related to iron metabolism beyond that from dietary iron intake. The exploratory analysis with iron status and hematologic traits, as well as the eQTL and functional annotations, adds to the biologic plausibility of the associations we observed. Our study also has limitations. The gene regions included in our analyses may not functionally influence the hepcidin pathway gene of interest but may be of functional importance for other nearby genes not part of the hepcidin pathway. Additional functional studies are needed to verify that observed associations represent a biologic relationship between the hepcidin-regulating iron metabolism pathway and PDAC. We do not have sufficient dietary data or power to evaluate pathway subgroup analyses stratified by iron or meat intake. The strongest pathway association was observed in the full set of cases and controls. Extreme care is warranted in interpretation of associations in subgroups (i.e., total meats and iron intakes, women, diagnosed at age >60 y, and BMI >30) because subgroup associations are known to be unreliable and underpowered: Spurious subgroup effects could be identified when none exist. Our analysis does not include less common pathogenic variants known to play a role in hereditary hemochromatosis; however, it does utilize common variants within the same genes. Individuals in our study were of European ancestry and most were aged >50 y; therefore, our findings may not be generalizable to other ancestral groups or younger individuals.

In conclusion, our results using common variants from GWAS support the hypothesis that the hepcidin-regulating iron metabolism pathway based on genes involved in iron sensing and regulation of dietary iron absorption is associated with PDAC. Further epidemiologic and experimental studies are needed to confirm our findings and to better understand the biologic mechanisms contributing to PDAC etiology.

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The authors' responsibilities were as follows—SJS and RZS-S: designed the research; KY: provided statistical methods and advice on their application; WW: performed statistical analysis; BB, AAA, LEB-F., PMB, EJD, MD, SG, GGG, PJG, CK, LLM, REN, X-OS, SKVDE, KV, WZ, DA, GA, AB, SIB, LKB, PB, BBdM, JEB, SJC, CCC, LF, CSF, JMG, MGG, TH, PH, MMH, IH, EAH, RIH, VJ, RCK, I-ML, NM, RLM, ALO, UP, MP, NR, MBS, HDS, DTS, IMT, JW-W, NW, E White, LRW, HY, AZ-J., PK, DL, GMP, BMW, HAR, LTA, APK, and RZS-S: provided essential reagents or provided essential materials; SJ-S, KY, and RZS-S: wrote the manuscript; SJ-S and RZS-S: had primary responsibility for final content; and all authors: contributed substantive interpretation of and editorial comments on the manuscript drafts and reviewed, read, and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

Data described in the manuscript and code book are available on dbGAP. Biomedical research scientists from recognized research institutions can request the data from dbGAP as bona fide researchers.

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