American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
editorial@hematology.org

Iron, haemochromatosis genotypes, and risk of infections: a cohort study of 142,188 general population individuals

Tracking no: BLD-2023-022235R2

Mathis Mottelson (Copenhagen University Hospital - Rigshospitalet, Denmark) Andreas Glenthøj (Danish Red Blood Cell Center, Department of Hematology, Copenhagen University Hospital - Rigshospitalet, Denmark) Børge Nordestgaard (University of Copenhagen, Denmark) Christina Ellervik (University of Copenhagen, Denmark) Jesper Petersen (Copenhagen University Hospital, Rigshospitalet, Denmark) Stig Bojesen (Copenhagen University Hospital, Denmark) Jens Helby (The Copenhagen General Population Study, Copenhagen University Hospital - Herlev and Gentofte, Herlev, Denmark, Denmark)

Abstract:
It is unclear whether risk of infection is increased in individuals with hereditary haemochromatosis and in individuals with low or high plasma iron, transferrin saturation, or ferritin. Therefore, we tested whether high and low iron, transferrin saturation, and ferritin are associated with risk of infections observationally and genetically through HFE genotypes. We studied 142,188 Danish general population individuals. Iron, transferrin saturation, and ferritin were measured in 136,656, 136,599, and 38,020 individuals, respectively. HFE was genotyped for C282Y and H63D in 132,542 individuals. Median follow-up after study enrolment was 8 years(range:0-38years) for hospital and emergency room admissions with infections(n=20,394 individuals) using the National Patient Register, covering all Danish hospitals. Hazard ratios for any infection were 1.20(95%CI:1.12-1.28) and 1.14(1.07-1.22) in individuals with plasma iron{less than or equal to}5th or {greater than or equal to}95th percentile compared to individuals with iron from 26th-74th percentiles. Findings for transferrin saturation were similar, while infection risk was not increased in individuals with ferritin{less than or equal to}5th or {greater than or equal to}95th percentile. Hazard ratios in C282Y homozygotes versus non-carriers were 1.40(1.16-1.68) for any infection, 1.69(1.05-2.73) for sepsis, and 2.34(1.41-3.90) for death from infectious disease. Risk of infection was increased in C282Y homozygotes with normal plasma iron, transferrin saturation, or ferritin, and in C282Y homozygotes without liver disease, diabetes, and/or heart failure. In summary, low and high plasma iron and transferrin saturation were independently associated with increased infection risk. C282Y homozygotes had increased risk of any infection, sepsis, and death from infections. Even C282Y homozygotes with normal iron, transferrin saturation, or ferritin, not currently recommended for genotyping, had increased infection risk.

Conflict of interest: COI declared - see note

COI notes: All authors declare: no support from any commercial organisation for the submitted work; AG has done consultancy for Pharmacosmos A/S, received research funding from Sanofi A/S, and received research funding and payment for consultancy/advisory board work from Novo Nordisk A/S. All other authors declare no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work. This work was supported by research grants from the Capital Region of Denmark, Karla og Verner Sørensens Almennyttige Fond, Beckett-Fonden, and the Independent Research Fund Denmark. The Copenhagen General Population Study and the Copenhagen City Heart Study are supported by the Danish Heart Foundation and Copenhagen University Hospital - Herlev and Gentofte. Christina Ellervik is partly funded by the Laboratory Medicine Endowment Fund of Boston Children's Hospital. The funders had no input in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the article for publication. The researchers acted independently from the study sponsors in all aspects of this study.

Preprint server: No;

Author contributions and disclosures: Conception and design: M. Mottelson, A. Glenthøj, J. Helby. Collection of data and assembly of databases: M. Mottelson, B.G. Nordestgaard, C. Ellervik, S.E. Bojesen, J. Helby. Analysis and interpretation of data: M. Mottelson, A. Glenthøj, B. G. Nordestgaard, C. Ellervik, J. Petersen, S. E. Bojesen, J. Helby. Writing of manuscript drafts: M. Mottelson, J. Helby. Manuscript revision, full access to all data, responsibility, and final approval of manuscript: M. Mottelson, A. Glenthøj, B. G. Nordestgaard, C. Ellervik, J. Petersen, S. E. Bojesen, J. Helby. M. Mottelson and J. Helby accessed and verified the underlying data reported in the manuscripts.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: Supplementary data (e.g., technical details, statistical code) can be made available from the corresponding author, please contact jens.helby.petersen.02@regionh.dk. Only through collaborative agreement or locally managed access, can further data be made available for additional analysis.

Clinical trial registration information (if any):

Title: Iron, haemochromatosis genotypes, and risk of infections: a cohort study of 142,188 general population individuals

Short title: Iron, HFE haemochromatosis, and infections

Authors: Mathis Mottelson (0000-0001-8736-2618)[1-3], Andreas Glenthøj (0000-0003-2082-0738)[1,2], Børge Grønne Nordestgaard (0000-0002-1954-7220)[2-5], Christina Ellervik (0000-0002-3088-4375)[2,6-8], Jesper Petersen (0000-0001-6116-5114)[1], Stig Egil Bojesen (0000-0002-4061-4133)[2-5], and Jens Helby (0000-0002-1876-8187)[1-3]

[1]Danish Red Blood Cell Centre, Department of Haematology, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark.

[2]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark.

[3]The Copenhagen General Population Study, Copenhagen University Hospital – Herlev and Gentofte, Herlev, Denmark

[4]Department of Clinical Biochemistry, Copenhagen University Hospital – Herlev and Gentofte, Herlev, Denmark

[5]The Copenhagen City Heart Study, Copenhagen University Hospital – Bispebjerg and Frederiksberg, Copenhagen, Denmark

[6]Department of Production, Research, and Innovation, Region Zealand, Sorø, Denmark.

[7]Department of Laboratory Medicine, Boston Children's Hospital, Boston, MA, USA.

[8]Department of Pathology, Harvard Medical School, Boston, MA, USA.

Statement of prior presentation

Presented in abstract form at the EHA2022 Hybrid Congress, Vienna, June 2022.

Corresponding author:

MD, PhD, Jens Helby, Department of Haematology, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

Phone: +45 35 45 20 84. E-mail: jens.helby.petersen.02@regionh.dk

Data Sharing

Supplementary data (e.g., technical details, statistical code) can be made available from the corresponding author, please contact jens.helby.petersen.02@regionh.dk. Only through collaborative agreement or locally managed access, can further data be made available for additional analysis.

Word count

Abstract: 250 words (max 250 words)

Full article: 4316 (max 4000 words)

Number of figures and tables: Tables: 1, Figures: 6 (max 7)

- Supplementary files: 1 (2836 words of Supplementary Methods, Supplementary Tables: 6, Supplementary Figures: 15)

Scientific category: Red cells, Iron, and Erythropoiesis

Key Points

- Haemochromatosis C282Y homozygotes had increased risk of any infection, and markedly increased risk of sepsis and death from infections

- C282Y homozygotes with normal iron, transferrin saturation, or ferritin, not currently recommended for genotyping, had high infection risk

Abstract

It is unclear whether risk of infection is increased in individuals with hereditary haemochromatosis and in individuals with low or high plasma iron, transferrin saturation, or ferritin. Therefore, we tested whether high and low iron, transferrin saturation, and ferritin are associated with risk of infections observationally and genetically through *HFE* genotypes. We studied 142,188 Danish general population individuals. Iron, transferrin saturation, and ferritin were measured in 136,656, 136,599, and 38,020 individuals, respectively. *HFE* was genotyped for C282Y and H63D in 132,542 individuals. Median follow-up after study enrolment was 8 years(range:0-38years) for hospital and emergency room admissions with infections(n=20,394 individuals) using the National Patient Register, covering all Danish hospitals. Hazard ratios for any infection were 1.20(95%CI:1.12-1.28) and 1.14(1.07-1.22) in individuals with plasma iron≤5th or ≥95th percentile compared to individuals with iron from 26th-74th percentiles. Findings for transferrin saturation were similar, while infection risk was not increased in individuals with ferritin≤5th or ≥95th percentile. Hazard ratios in C282Y homozygotes versus non-carriers were 1.40(1.16-1.68) for any infection, 1.69(1.05-2.73) for sepsis, and 2.34(1.41-3.90) for death from infectious disease. Risk of infection was increased in C282Y homozygotes with normal plasma iron, transferrin saturation, or ferritin, and in C282Y homozygotes without liver disease, diabetes, and/or heart failure. In summary, low and high plasma iron and transferrin saturation were independently associated with increased infection risk. C282Y homozygotes had increased risk of any infection, sepsis, and death from infections. Even C282Y homozygotes with normal iron, transferrin saturation, or ferritin, not currently recommended for genotyping, had increased infection risk.

Introduction

Iron is a life-essential micronutrient which is necessary for haemoglobin synthesis and function of immune cells but also a prerequisite for growth of microorganisms.[1,2] Serum iron parameters are often measured in routine blood samples where plasma iron and transferrin saturation indicate how much iron is available in the bloodstream, while plasma ferritin is a measure of total iron accumulated in the body.[3–5] It is unclear whether high levels of plasma iron, transferrin saturation, and/or ferritin increase susceptibility to infections, as recent studies have shown conflicting results.[3,5]

Individuals with the condition haemochromatosis have increased accumulation of iron in the body, which can lead to liver cirrhosis, hepatocellular cancer, diabetes mellitus, heart failure, and arthritis.[6,7] Hereditary haemochromatosis is most often caused by homozygosity or compound heterozygosity for single nucleotide polymorphism variants in the *HFE* haemochromatosis gene encoding the human homeostatic iron regulator protein. These variants are C282Y (rs1800562) and H63D (rs1799945), either as C282Y homozygosity (C282Y/C282Y) having a prevalence of 0.25-1.0% in Northern Europe or as compound heterozygosity (C282Y/H63D) with a prevalence of about 2% in Northern Europe.[8,9] On average, C282Y homozygotes have higher levels of plasma iron, transferrin saturation, and ferritin than non-carrier individuals,[10–13] but results on penetrance have varied substantially between studies, as clinical penetrance has been reported in between 1-60% of C282Y homozygous individuals.[7,9,14] In Northern Europe, 80-95% of individuals diagnosed with hereditary haemochromatosis are homozygous for the *HFE* C282Y variant.[11,15,16]

It is unclear if homozygosity and/or heterozygosity for the C282Y or H63D variants cause increased risk of infections. This question is important due to possible clinical consequences especially for C282Y homozygous individuals, but also because it offers a method to study potentially causal effects on risk of infections from iron accumulation.[17]

We tested the hypothesis that high and low plasma iron, transferrin saturation, and ferritin are associated with increased risk of infections observationally and genetically through *HFE* C282Y and H63D variants. For this purpose,

we studied 142,188 individuals from the white Danish general population. All individuals had blood samples drawn and were followed for hospitalization and death due to infections.

Methods

We studied 142,188 individuals from three cohort studies of the Danish general population: The Copenhagen City Heart Study, the Copenhagen General Population Study, and the Danish General Suburban Population Study. From the Copenhagen City Heart Study[18], we included 13,661 individuals examined between 1981-1983 or between 1991-1994. From the Copenhagen General Population Study[19], we included 108,061 individuals examined between 2003-2015. From the Danish General Suburban Population Study[20], we included 20,466 individuals examined between 2010-2013. Details on enrolment procedures and participation rates are described in the "Supplementary Methods". Age at enrolment was 20-100 years. At the day of enrolment, all individuals had blood samples drawn, filled out a questionnaire regarding health and lifestyle, and had a physical examination performed. Study procedures were similar for the three general population studies. More than 99% of individuals were white and of Danish descent. Individuals were only included into one of the three studies, and none were lost to follow-up. The studies were approved by Danish ethical committees and all participants provided written, informed consent.

Covariates

Covariates associated with risk of infection and/or with levels of plasma iron, transferrin saturation, or ferritin were chosen based on an *a priori* literature review.[21] Information on age and sex was obtained from the Danish Civil Registration System. Smoking status, cumulative smoking in packyears, alcohol intake, and menopausal status were derived from the questionnaire, while body mass index was from the physical examination (details in "Supplementary methods"). C-reactive protein levels were measured using high sensitivity standard laboratory assays.

Plasma iron, transferrin saturation, and ferritin

All individuals had blood samples drawn at the day of study enrolment used for measuring iron, transferrin saturation, and ferritin. In the Copenhagen City Heart Study and the Copenhagen General Population Study, plasma iron and transferrin were measured using a Konelab autoanalyzer (ThermoFisher assays "IRON" and "TRANSFERRIN"), while ferritin was measured using an Advia Centaur (Siemens assay "FER"). In the Danish General Suburban Population Study, iron, transferrin, and ferritin were measured using a Cobas 6000 (Roche assays "IRON2", "TRANS", and "Ferritin").

For a total of 32,141 individuals, a second blood sample for a repeat measurement of iron and/or transferrin saturation was obtained at a median of 10 years after the first blood sample as described in "Supplementary Methods" along details on sample storage, calibration, and quality control.

Genotypes

We genotyped the two haemochromatosis variants C282Y and H63D in the *HFE* gene, both known to increase levels of iron, transferrin saturation and ferritin.[6] For details on genotyping and Hardy-Weinberg equilibrium see "Supplementary Methods".

Infectious disease and vital status

All hospital inpatient admissions in Denmark since 1977 and all emergency room visits and outpatient visits since 1994 have been registered in the Danish National Patient Register.[22] We obtained information from this register on each individual regarding all inpatient admissions with an infectious disease as the main diagnosis from January 1st, 1977, until December 31st, 2018. Similarly, we obtained information on all emergency room visits with an infectious disease as the main diagnosis from January 1st, 1994, until December 31st, 2018. Categorization of infectious disease hospitalizations are described in "Supplementary Methods" and Supplementary Table S1. Information on bloodstream infections from January 1st, 2010 to December 31st, 2019 was retrieved from the national Danish Microbiology Database[23] as described in "Supplementary Methods".

We have previously ascertained validity of infectious disease diagnoses from the Danish National Patient Register through review of detailed clinical information from hospital charts on 141 admissions coded as infectious diseases. In 139 of 141 admissions (99%), hospital charts documented relevant signs and symptoms of infection, a positive culture from a sterile site or relevant specimen, and/or treatment with antibiotics.[18]

Information on cause of death until December 31st, 2018, was retrieved from the national Danish Cause of Death Register containing all diagnoses that a physician listed on the death certificate for all deaths occurring in Denmark from 1970 and onwards.[24] Death from infectious disease was defined as any death with an infection as the underlying cause of death, immediate cause of death, or the disease linking the underlying and immediate cause of death. Information about vital status and emigration until December 31st, 2019, was retrieved from the national Danish Civil Registration System.

Comorbidities

Non-infectious comorbidities can be associated with levels of plasma iron, transferrin saturation, and ferritin, but also with risk of infection.[25,26] Therefore, we retrieved information on comorbidities at study enrolment from the Danish National Patient Register. Comorbidities were defined using the Charlson comorbidity index (see "Supplementary Methods").

Statistical analysis

Statistical analyses were performed using Stata17.0. All statistical tests were two-sided. Risk of infection and risk of death from infection was modelled by Cox proportional hazards regression for the main analyses while Fine-Gray competing risk regression with risk of infection as main endpoint and death as a competing risk were performed as supplementary analyses.[27] We adjusted for age by using left-truncated age (time since birth with delayed entry) as time scale, which is often recommended for studies of the general population since the date of study enrolment is

largely chosen at random, meaning that age is generally a stronger predictor for risk of future disease than time since study enrolment.[28,29]

When analysing risk of hospitalization with infection according to levels of iron, transferrin saturation, and ferritin, follow-up began at the date of study enrolment. We divided individuals into five categories of measured iron, transferrin saturation, and ferritin, with focus on extreme high and extreme low levels:0^{th}-5^{th}, 6^{th}-25^{th}, 26^{th}-74^{th}, 75^{th}-94^{th}, and 95^{th}-100^{th} percentile. The 26^{th}-74^{th} percentile was used as the reference group. When analysing risk of hospitalization with infection according to *HFE* genotype, follow-up began at age 20 or January 1^{st}, 1977, whichever came last. When analysing risk of infectious disease death according to levels of iron, transferrin saturation, ferritin, or *HFE* genotype, follow-up began at the date of study enrolment. Further details on follow-up (including follow-up for the analysis on risk of bloodstream infections), statistical models, and handling of missing values are described in "Supplementary Methods" and Supplementary Figure S1. Infections before start of follow-up were ignored in all analyses.

The studies were approved by Danish ethical committees (De Videnskabsetiske Komit�er for K�benhavns og Frederiksberg kommuner, Region Sj�llands forskningsfortegnelse). All participants provided written, informed consent.

Results

Table 1 presents baseline characteristics of the 142,188 general population individuals according to study cohort, while baseline characteristics according to levels of plasma iron, transferrin saturation, and ferritin are presented in Supplementary Tables S2-S4. From study enrolment until end of follow-up (median:8 years, range:0-38 years) 20,394 individuals were hospitalized due to infections. The rate of infections was 1,144 infections per 100,000 individuals per year in the first year after study enrolment with an increasing trend until the 36^{th} year after study enrolment where the rate peaked at 5,055 infections per 100,000 individuals per year (Supplementary Figure S2).

Risk of infection according to levels of iron, transferrin saturation, and ferritin

Kaplan-Meier curves for risk of any infection according to levels of iron, transferrin saturation, and ferritin are shown in Figure 1. After multivariable adjustment, low plasma iron was associated with increased risk of any infection with a hazard ratio of 1.20 (95% confidence interval [95%CI] 1.12-1.28) for individuals with iron at or below the 5th percentile (7.1 µmol/l) when compared to individuals with iron between the 26th-74th percentile (Figure 2). High plasma iron was also associated with increased risk of any infection with a hazard ratio of 1.14 (1.07-1.22) for iron at or above the 95th percentile (22.5 µmol/l) vs. between the 26th-74th percentiles. Similarly, both low transferrin saturation (hazard ratio 1.18;95%CI 1.10-1.26 for transferrin saturation at or below the 5th percentile [11%]) and high transferrin saturation (hazard ratio 1.10;1.03-1.17 for transferrin saturation at or above the 95th percentile [39%]) were associated with increased risk of any infection. For ferritin, we found no association between low (≤5th percentile) or high (≥95th percentile) levels and risk of any infection (Figure 2). Results were similar to those described above when performing competing risk analysis for risk of any infection according to levels of iron, transferrin saturation, and ferritin with death as a competing risk (Figure 2). Likewise, results were similar to those presented in Figure 2 when additionally including household income and education level in the multivariable adjusted model or when only including individuals who were non-carriers for both C282Y and H63D (non-carrier/non-carrier)(Supplementary Table S5).

As levels of iron and transferrin saturation at or below the 5th percentile are common in acute infections, chronic inflammatory disorders, and other comorbidities, we performed separate analyses on risk of any infection according to levels of iron, transferrin saturation, and ferritin excluding individuals who had any infection within the first 180 days after study enrolment and excluding individuals who at study enrolment had anaemia, neutropenia, any comorbidity, or a C-reactive protein level above 10 mg/l (Supplementary Table S5). All these analyses gave results similar to those presented in Figure 2.

When stratified according to follow-up intervals, risk estimates for any infection were similar for the first 5 years

after study enrolment and for the time interval from 5 years after study enrolment and onwards (Supplementary Figure S3), indicating that the increased risk was not likely caused by latent infections already present at study enrolment. Since non-transferrin bound iron is often considered to occur when transferrin saturation exceeds 70%[30], we also examined risk of any infection after excluding the 254 individuals who had a transferrin saturation at or above 70% at day of study enrolment, which gave results similar to those presented in Figure 2 (Supplementary Table S5). When comparing the 254 individuals with transferrin saturation at or above 70% to the 58,997 individuals with transferrin saturation between the 26th-74th percentile, the hazard ratio for any infection was 1.23 (95%CI:0.91-1.65). These results indicate that non-transferrin bound iron is not likely the primary cause of the increased risk of any infection in individuals with high iron or high transferrin saturation.

As levels of iron and transferrin saturation may change over time[31,32], Figure 3 shows changes in iron and transferrin saturation in 32,141 individuals who had a second blood sample obtained for repeat measurements after a median of 10 years. Among these, 26,240 individuals had a repeat measurement performed before end of follow-up for infectious disease hospitalizations on December 31st, 2018. When studying risk of any infection according to the first measurements of iron and transferrin saturation only in the individuals with repeat measurements available (Supplementary Figure S4), risk estimates were generally similar to those observed for the whole study population (Figure 2), but with substantially lower statistical power and therefore wider confidence intervals due to the limited number of individuals with repeat measurements available. Likewise, when studying risk of any infection according to the second (repeat) measurements of iron and transferrin saturation, risk estimates were generally similar to those observed when using the first measurement (Supplementary Figure S4 and Figure 2).

As more than 90% of infection related deaths are due to pneumonia or sepsis[19], results on risk of pneumonia and sepsis are presented in detail (Figure 4). Both low iron (hazard ratio 1.20;95%CI 1.10-1.32) and high iron (1.22;1.11-1.34) were associated with increased risk of pneumonia. Likewise, low transferrin saturation (1.20;1.09-1.32) and high transferrin saturation (1.11;1.01-1.22) were associated with increased risk of pneumonia.
For sepsis, both low iron (hazard ratio 1.18;95%CI 1.01-1.38) and high iron (1.38;1.20-1.58) were associated with

increased risk (Figure 4). Low transferrin saturation was not associated with risk of sepsis (1.12;0.95-1.32), but high transferrin saturation was associated with increased risk of sepsis (1.27;1.11-1.46). When studying risk of any bloodstream infection, defined as any episode where one or more microorganisms grew in a blood culture, results were similar to those found for sepsis with an increased risk of any bloodstream infection observed in individuals with low iron (1.23;1.05-1.44), high iron (1.26;1.08-1.46) and high transferrin saturation (1.24;1.07-1.44)(Supplementary Figure S5). The increased risk of bloodstream infections was primarily due to gram-positive bacteria with hazard ratios of 1.45 (95%CI:1.20-1.77) for low iron, 1.31 (1.07-1.59) for high iron, 1.27 (1.02-1.58) for low transferrin saturation and 1.38 (1.13-1.67) for high transferrin saturation (Supplementary Figure S6). Levels of iron, transferrin saturation and ferritin were not associated with risk of gram-negative bloodstream infections (Supplementary Figure S6).

When investigating risk of other specific types of infection, low iron was associated with increased risk of urinary tract infection (1.20;1.06-1.36) and skin infection (1.18;1.02-1.36). For transferrin saturation, low levels were associated with increased risk of urinary tract infection (1.18;1.03-1.34), while high levels were associated with increased risk of diarrhoeal disease (1.27;1.04-1.56)(Supplementary Figure S7). Risk estimates for any infection, pneumonia, and sepsis according to low or high levels of iron, transferrin saturation, and ferritin were similar for men and women with no indication of interaction between sex and any of the three biomarkers on risk of any infection, pneumonia, or sepsis (Supplementary Figure S8).

When examining risk of death from infectious disease, low iron (1.31;1.14-1.52) and low transferrin saturation (1.31;1.12-1.53) were associated with increased risk, while risk was not increased in individuals with high iron (1.04;0.88-1.23), high transferrin saturation (1.09;0.93-1.27), high ferritin (0.95;0.74-1.22), or low ferritin (1.28;0.98-1.68)(Supplementary Figure S9). When stratified according to follow-up intervals, risk estimates for death from infectious disease were similar for the first 5 years after study enrolment and for the time interval from 5 years after study enrolment and onwards (Supplementary Figure S9).

Risk of infections according to haemochromatosis genotypes

Among 132,542 individuals genotyped for *HFE* C282Y and H63D, 422 individuals were homozygous for the C282Y variant (228 women and 194 men). Supplementary Table S6 presents baseline characteristics according to genotypes, showing potential associations with genotype for year of birth (P for trend=0.03) and neutrophil count (P for trend=0.04), however, when adjusting for 12 multiple comparisons using the Bonferroni method, these findings were no longer significant at the P<0.05 level (required P-value < 0.004=0.05/12). Follow-up for the analysis on risk of infections according to haemochromatosis genotypes began at age 20 or January 1st, 1977, whichever came last (illustrated in Supplementary Figure S1). During follow-up (median:39 years, range:0-42 years), 29,936 of the genotyped individuals were hospitalized due to infections, of which 14,747 were hospitalized before study enrolment and 15,189 were hospitalized after.

Individuals with C282Y homozygosity (C282Y/C282Y) had increased risk of any infection (hazard ratio 1.40;1.16-1.68) when compared to individuals who were non-carrier for both C282Y and H63D (non-carrier/non-carrier)(Figure 1 and Figure 5). Results were similar when performing competing risk analysis for risk of any infection with death as a competing risk (hazard ratio 1.36;1.13-1.64 for C282Y homozygotes vs. non-carriers)(Supplementary Figure S10). For pneumonia, the hazard ratio was 1.26 (0.92-1.72) in C282Y homozygotes. Risk of sepsis was markedly increased in C282Y homozygotes with a hazard ratio of 1.69 (1.05-2.73). For other specific types of infection in C282Y homozygotes, risk was only increased at the P<0.05 level for skin infection (hazard ratio 1.50;95%CI:1.10-2.03;P=0.009), although pneumonia (1.26;0.92-1.72;P=0.15), urinary tract infection (1.20;0.78-1.84;P=0.41), and endocarditis (2.98;0.95-9.30;P=0.06) may also contribute to the increased risk of any infection (Supplementary Figure S11). Risk of any bloodstream infection was not significantly increased at the P<0.05 level for C282Y homozygotes (hazard ratio 1.31;0.72-2.36;P=0.38), however, statistical power was limited, as only 11 C282Y homozygotes had bloodstream infections (Supplementary Figure S12). When stratified by sex, risk estimates for any infection were more pronounced for C282Y homozygous women than men (hazard ratio 1.68;95%CI 1.32-2.14 for C282Y homozygous women compared to non-carrier women;hazard ratio 1.15;95%CI 0.86-1.53 for homozygous men

compared to non-carrier men;P for interaction with sex=0.03)(Supplementary Figure S13). Risk of any infection (0.99;0.90-1.08) was not increased in compound heterozygous individuals (C282Y/H63D)(Figure 5).

Clinical guidelines from several countries including Denmark and The United Kingdom recommend testing patients for the haemochromatosis variants C282Y and H63D only when both transferrin saturation and ferritin level are increased.[8,10,11,33] If either transferrin saturation or ferritin is normal, testing for haemochromatosis is not usually recommended. Therefore, we examined risk of infection in C282Y homozygotes not currently recommended for haemochromatosis genotype testing by stratifying C282Y homozygotes by levels of iron, transferrin saturation, and ferritin at study enrolment. Surprisingly, risk of any infection was increased in C282Y homozygotes with normal plasma iron (hazard ratio 1.50;95%CI 1.23-1.83 for C282Y homozygotes with normal iron vs. non-carrier individuals with normal iron), normal transferrin saturation (hazard ratio 1.47;1.03-2.10), or normal ferritin (2.39;1.29-4.45)(Figure 6).

A total of 89 C282Y homozygotes had a second blood sample for a repeat measurement of iron and transferrin saturation obtained at a median of 10 years after the first blood sample (which was obtained at study enrolment). Among the 89 C282Y homozygotes with repeat blood samples, 76 had a normal iron level at study enrolment and 88.2% (67 individuals) of these still had a normal iron level after a median of 10 years. Likewise, among 24 C282Y homozygotes with normal transferrin saturation at study enrolment, 62.5% (15 individuals) still had normal transferrin saturation after a median of 10 years. No individuals had repeat measurements of ferritin performed.

Risk of any infection was increased in C282Y homozygotes without liver disease, diabetes, or heart failure diagnosed at any time before or after study enrolment (hazard ratio 1.32;95%CI 1.07-1.64)(Supplementary Figure S14). When stratifying C282Y homozygotes according to whether or not they had been diagnosed with haemochromatosis at any time before or after study enrolment, we found similar risk estimates for any infection in C282Y homozygotes

diagnosed with haemochromatosis (hazard ratio 1.43;95%CI 0.94-2.17 when compared to non-carrier individuals) and in C282Y homozygotes never diagnosed with haemochromatosis (1.39;1.13-1.71)(Supplementary Figure S15).

To examine whether C282Y homozygotes also had increased risk of fatal infections, we studied risk of death from infectious disease according to haemochromatosis genotype. After age and sex adjustment, individuals with C282Y homozygosity had increased risk of death from infectious disease compared to non-carrier individuals (hazard ratio 2.34;95%CI 1.41-3.90)(Figure 5).

Discussion

In this study of 142,188 individuals from the general population, we found that high and low plasma iron and high and low transferrin saturation were associated with increased risk of any infection. Further, individuals with haemochromatosis C282Y homozygosity had increased risk of any infection and markedly increased risk of sepsis and death from infectious disease. Finally, despite normal levels of iron, transferrin saturation, or ferritin, C282Y homozygotes had high risk of any infection. These are novel findings.

The increased risk of infection in individuals with both low and high iron indicates a U-shaped relationship between iron available in the bloodstream and risk of infections. This could hypothetically be explained by increased proliferation of invading pathogens in iron-rich environments[34] and decreased proliferation in individuals with low-normal levels of iron essential for various biochemical processes in the pathogen.[2,34] Thus decreasing levels of iron could possibly reduce risk of infection until a certain low level where iron dependent lymphocyte and/or neutrophil proliferation and activation becomes impaired, theoretically increasing susceptibility to infections.[2,35–39]

Our finding that high levels of iron and high transferrin saturation were associated with increased risk of infections

including increased risk of sepsis and bloodstream infections is novel. Our results contrasts a Norwegian study of 61,852 individuals from the general population of whom 1,738 had bloodstream infections[3], as the Norwegian study observed a high risk of bloodstream infections in individuals with low levels of plasma iron or transferrin saturation, but not in individuals with high levels. The different results between the Danish and Norwegian studies may be explained by higher statistical power in our study of 142,188 individuals, of which 20,394 were hospitalized with any infection and 3,711 were hospitalized with sepsis after study enrolment.

Our finding that haemochromatosis C282Y homozygotes are at increased risk of any infection and at markedly increased risk of sepsis and death from infectious disease is likewise novel. That said, this finding is partly supported by a study based on the United Kingdom (UK) Biobank, examining risk of pneumonia in 451,243 general population individuals genotyped for C282Y and H63D.[13] In the UK study, increased risk of pneumonia was observed among men homozygous for C282Y, but not among women, which has been proposed to be due to menstrual bleeding in women leading to less accumulation of iron.[13,40] In contrast, when stratifying the analyses by sex, our study found lower risk estimates for any infection in C282Y homozygous men than in homozygous women, whereas we found similarly increased risk of sepsis in C282Y homozygous men and women.

Our novel findings that even C282Y homozygous individuals with normal levels of iron, transferrin saturation, or ferritin had increased risk of infection and that risk of infection was also high in C282Y homozygotes not diagnosed with organ damage (liver disease, diabetes, or heart failure) potentially caused by iron overload, could perhaps influence decisions on which individuals should be tested for hereditary haemochromatosis. Clinical guidelines from several countries recommend testing patients for the *HFE* variants C282Y and H63D only when both transferrin saturation and ferritin level is increased.[8,10,33] Therefore, our finding that even C282Y homozygous individuals with normal levels of iron, transferrin saturation, or ferritin had increased risk of infection indicate that C282Y homozygotes not currently recommended for genetic testing had increased risk of infection.

Mechanistically, the increased infection risk in C282Y homozygotes with normal levels of iron, transferrin, or ferritin, and the especially high risk in C282Y homozygous women could hypothetically be explained by the lower levels of the hepcidin hormone in C282Y homozygotes. Hepcidin is the major hormone regulating iron uptake from the intestine, but it also carries antimicrobial properties.[1,41] Impairment in inducing hepcidin through the *HFE* dependent pathway in C282Y homozygotes could infer suboptimal response to infections regardless of each individual's steady-state level of iron, transferrin saturation, or ferritin. Other potential mechanisms include *HFE* variants causing altered intracellular iron levels in macrophages, with potential importance in intracellular infections[42], or the increased infection risk might be due to a linked variant, as the genomic neighborhood of *HFE* contains several immune-related genes encoding antigen-presenting molecules and cytokines, including TNF-alpha[43–45]. However, we are not aware of any specifically linked variants and all the above-mentioned hypothetical mechanisms need further investigation.

Strengths of this study include the large cohort, our comprehensive data on health and lifestyle and the nationwide Danish registries without any losses to follow-up enabling detailed information on comorbidities and infectious disease hospitalizations. Among the limitations of our study is the observational nature when examining risk of infection according to plasma iron, transferrin saturation and ferritin. As maximum follow-up after study enrolment was 38 years, levels of iron, transferrin saturation, and ferritin for each individual could have changed between time of measurement and time of infection, which represents a potential weakness of the study. Further, although the multivariable adjusted model included C-reactive protein and comorbidities and we performed several stratified analyses excluding individuals with indices of conditions affecting iron parameters or risk of infection, we cannot rule out residual confounding, which limits interpretation of causality. Importantly, however, the association between haemochromatosis C282Y genotype and risk of infections is likely causal, since genes are assorted at random in the gamete state ensuing random distribution of confounding factors, making reverse causality impossible and confounding unlikely.

Further studies are needed to elucidate whether prophylactic interventions could limit severe infections in C282Y homozygotes. For example, wider use of pneumococcal vaccination or wider use of antibiotic treatment in case of fever may hypothetically limit risk of sepsis and death from infections in C282Y homozygotes; however, this is speculative and further studies are needed. The need for further studies is underlined by our finding that individuals with C282Y homozygosity diagnosed with haemochromatosis at a hospital at any time before or after study enrolment had similar risk of infection as C282Y homozygotes never diagnosed with haemochromatosis. Although the effect of therapeutic phlebotomy cannot directly be assessed in this observational study, our results might imply that the current treatment of haemochromatosis, where phlebotomy is performed to reduce overall iron storage, may possibly not sufficiently reduce infection risk.

In summary, low and high iron and transferrin saturation were associated with increased infection risk. C282Y homozygotes had increased risk of any infection, sepsis and death from infections. Even C282Y homozygotes with normal iron, transferrin saturation, or ferritin, not currently recommended for genotyping, had increased infection risk.

Acknowledgements

This study was conducted using data from the Copenhagen City Heart Study, the Copenhagen General Population Study, and the Danish General Suburban Population Study. We thank all participants and staff of the studies. We also thank Professor Jørgen Kurtzhals from the University of Copenhagen for his invaluable assistance in obtaining essential microbiology data for this study. Furthermore, we would like to express our gratitude to the 3 anonymous reviewers for their helpful and constructive suggestions during the review process.

Authorship contribution:

Conception and design: M. Mottelson, A. Glenthøj, J. Helby.

Collection of data and assembly of databases: M. Mottelson, B.G. Nordestgaard, C. Ellervik, S.E. Bojesen, J. Helby.

Analysis and interpretation of data: M. Mottelson, A. Glenthøj, B. G. Nordestgaard, C. Ellervik, J. Petersen, S. E. Bojesen, J. Helby.

Writing of manuscript drafts: M. Mottelson, J. Helby.

Manuscript revision, full access to all data, responsibility, and final approval of manuscript: M. Mottelson, A. Glenthøj, B. G. Nordestgaard, C. Ellervik, J. Petersen, S. E. Bojesen, J. Helby.

M. Mottelson and J. Helby accessed and verified the underlying data reported in the manuscripts.

Conflict of interest disclosure:

All authors declare: no support from any commercial organisation for the submitted work; AG has done consultancy for Pharmacosmos A/S, received research funding from Sanofi A/S, and received research funding and payment for consultancy/advisory board work from Novo Nordisk A/S. All other authors declare no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

This work was supported by research grants from the Capital Region of Denmark, Karla og Verner Sørensens Almennyttige Fond, Beckett-Fonden, and the Independent Research Fund Denmark. The Copenhagen General Population Study and the Copenhagen City Heart Study are supported by the Danish Heart Foundation and Copenhagen University Hospital - Herlev and Gentofte. Christina Ellervik is partly funded by the Laboratory Medicine Endowment Fund of Boston Children's Hospital. The funders had no input in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the article for publication. The researchers acted independently from the study sponsors in all aspects of this study.

Correspondence

MD, PhD, Jens Helby, Department of Haematology, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

Phone: +45 35 45 20 84. E-mail: jens.helby.petersen.02@regionh.dk

References

1. Ganz T. Iron and infection. *Int J Hematol*. 2018;107(1):7-15. doi:10.1007/s12185-017-2366-2

2. Drakesmith H, Prentice AM. Hepcidin and the iron-infection axis. *Science*. 2012;338(6108):768-772. doi:10.1126/science.1224577

3. Mohus RM, Paulsen J, Gustad L, et al. Association of iron status with the risk of bloodstream infections: results from the prospective population-based HUNT Study in Norway. *Intensive Care Med*. 2018;44(8):1276-1283. doi:10.1007/s00134-018-5320-8

4. Sazawal S, Black RE, Ramsan M, et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in. *Lancet*. 2006;367(9505):133-143.

5. Hamilton F, Mitchell R, Ahmed H, Ghazal P, Timpson NJ. An observational and Mendelian randomisation study on iron status and sepsis. *Sci Rep*. 2023;13(1):1-14. doi:10.1038/s41598-023-29641-6

6. Bomford A. Genetics of haemochromatosis. *Lancet*. 2002;360(9346):1673-1681. doi:10.1016/S0140-6736(02)11607-2

7. Allen KJ, Gurrin LC, Constantine CC, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. *N Engl J Med*. 2008;358(3):221-230. doi:10.1056/NEJMoa073286

8. Fitzsimons EJ, Cullis JO, Thomas DW, Tsochatzis E, Griffiths WJH. Diagnosis and therapy of genetic haemochromatosis (review and 2017 update). *Br J Haematol*. 2018;181(3):293-303. doi:10.1111/bjh.15164

9. Zoller H, Henninger B. Pathogenesis, Diagnosis and Treatment of Hemochromatosis. *Dig Dis*. 2016;34(4):364-373. doi:10.1159/000444549

10. Girelli D, Busti F, Brissot P, Cabantchik I, Muckenthaler MU, Porto G. Hemochromatosis classification: update and recommendations by the BIOIRON Society. *Blood*. 2022;139(20):3018-3029. doi:10.1182/blood.2021011338

11. Zoller H, Schaefer B, Vanclooster A, et al. EASL Clinical Practice Guidelines on haemochromatosis. *J Hepatol*. 2022;77(2):479-502. doi:10.1016/j.jhep.2022.03.033

12. Bacon BR, Adams PC, Kowdley K V., Powell LW, Tavill AS. Diagnosis and management of hemochromatosis: 2011 Practice Guideline by the American Association for the Study of Liver Diseases. *Hepatology*. 2011;54(1):328-343. doi:10.1002/hep.24330

13. Pilling LC, Tamosauskaite J, Jones G, et al. Common conditions associated with hereditary haemochromatosis genetic variants: Cohort study in UK Biobank. *BMJ*. 2019;364. doi:10.1136/bmj.k5222

14. Grosse SD, Morris JM, Khoury MJ. Disease-related conditions in relatives of patients with hemochromatosis. *N Engl J Med*. 2001;344(19):1477-1478. doi:10.1056/NEJM200105103441914

15. Pilling LC, Atkins JL, Melzer D. Genetic modifiers of penetrance to liver endpoints in HFE hemochromatosis: Associations in a large community cohort. *Hepatology*. 2022;(May):1735-1745. doi:10.1002/hep.32575

16. Merryweather-Clarke AT, Pointon JJ, Jouanolle AM, Rochette J, Robson KJH. Geography of HFE C282Y and H63D mutations. *Genet Test*. 2000;4(2):183-198. doi:10.1089/10906570050114902

17. Lawlor DA, Harbord RM, Sterne JAC, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. 2008;27(8):1133-1163. doi:10.1002/sim.3034

18. Benfield T, Jensen JS, Nordestgaard BG. Influence of diabetes and hyperglycaemia on infectious disease hospitalisation and outcome. *Diabetologia*. 2007;50(3):549-554. doi:10.1007/s00125-006-0570-3

19. Helby J, Nordestgaard BG, Benfield T, Bojesen SE. Shorter leukocyte telomere length is associated with higher risk of infections: a prospective study of 75,309 individuals from the general population. *Haematologica*. 2017;102(8):1457-1465. doi:10.3324/haematol.2016.161943

20. Sørensen AL, Hasselbalch HC, Nielsen CH, Poulsen HE, Ellervik C. Statin treatment, oxidative stress and inflammation in a Danish population. *Redox Biol*. 2019;21:101088. doi:10.1016/j.redox.2018.101088

21. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med*. 2005;352(10):1011-1023. doi:10.1056/NEJMra041809

22. Lynge E, Sandegaard JL, Rebolj M. The Danish National Patient Register. *Scand J Public Health*. 2011;39(7

Suppl):30-33. doi:10.1177/1403494811401482

23. Voldstedlund M, Haarh M, Mølbak K. The danish microbiology database (MIBA) 2010 to 2013. *Eurosurveillance*. 2014;19(1):20667. doi:10.2807/1560-7917.ES2014.19.1.20667

24. Helweg-Larsen K. The Danish Register of Causes of Death. *Scand J Public Health*. 2011;39(7 Suppl):26-29. doi:10.1177/1403494811399958

25. Jehn ML, Guallar E, Clark JM, et al. A prospective study of plasma ferritin level and incident diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Epidemiol*. 2007;165(9):1047-1054. doi:10.1093/aje/kwk093

26. Kim EJ, Ha KH, Kim DJ, Choi YH. Diabetes and the Risk of Infection: A National Cohort Study. *Diabetes Metab J*. 2019;43(6):804-814. doi:10.4093/dmj.2019.0071

27. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. *J Am Stat Assoc*. 1999;94(446):496-509. doi:10.1080/01621459.1999.10474144

28. Thiébaut ACM, Bénichou J. Choice of time-scale in Cox's model analysis of epidemiologic cohort data: A simulation study. *Stat Med*. 2004;23(24):3803-3820. doi:10.1002/sim.2098

29. Kleinbaum DG, Klein M. *Survival Analysis: A Self-Learning Text*. Third Edit. Springer New York; 2012.

30. Silva AMN, Rangel M. The (Bio)Chemistry of Non-Transferrin-Bound Iron. *Molecules*. 2022;27(6):1-14. doi:10.3390/molecules27061784

31. Adams PC, Jeffrey G, Ryan J. Haemochromatosis. *Lancet*. 2023;401(10390):1811-1821. doi:10.1016/S0140-6736(23)00287-8

32. Acton RT, Snively BM, Barton JC, et al. A genome-wide linkage scan for iron phenotype quantitative trait loci: The HEIRS family study. *Clin Genet*. 2007;71(6):518-529. doi:10.1111/j.1399-0004.2007.00804.x

33. Schiødt F V, Junker A, Magnussen K, Milman NT, Nathan T, Sandahl TD. National guidelines on haemochromatosis from the Danish Society of Gastroenterology and Hepatology (HFE-Hæmokromatose. Udredning, diagnostik og behandling). https://dsgh.dk/wp-content/uploads/2022/06/HFE-

Haemokromatose.pdf.

34. Schade AL, Caroline L. RAW HEN EGG WHITE AND THE ROLE OF IRON IN GROWTH INHIBITION OF SHIGELLA DYSENTERIAE, STAPHYLOCOCCUS AUREUS, ESCHERICHIA COLI AND SACCHAROMYCES CEREVISIAE. *Science*. 1944;100(2584):14-15. doi:10.1126/science.100.2584.14

35. Weiss G, Ganz T, Goodnough LT. Anemia of inflammation. *Blood*. 2019;133(1):40-50. doi:10.1182/blood-2018-06-856500

36. Jabara HH, Boyden SE, Chou J, et al. A missense mutation in TFRC, encoding transferrin receptor 1, causes combined immunodeficiency. *Nat Genet*. 2016;48(1):74-78. doi:10.1038/ng.3465

37. Frost JN, Tan TK, Abbas M, et al. Hepcidin-Mediated Hypoferremia Disrupts Immune Responses to Vaccination and Infection. *Med*. 2021;2(2):164-179.e12. doi:10.1016/j.medj.2020.10.004

38. Frost JN, Wideman SK, Preston AE, et al. Plasma iron controls neutrophil production and function. *Science (80-)*. 2022;8(40):1-12. doi:10.1126/sciadv.abq5384

39. Aljohani AH, Al-Mousa H, Arnaout R, et al. Clinical and Immunological Characterization of Combined Immunodeficiency Due to TFRC Mutation in Eight Patients. *J Clin Immunol*. 2020;40(8):1103-1110. doi:10.1007/s10875-020-00851-1

40. Atkins JL, Pilling LC, Masoli JAH, et al. Association of Hemochromatosis HFE p.C282Y Homozygosity with Hepatic Malignancy. *JAMA - J Am Med Assoc*. 2020;324(20):2048-2057. doi:10.1001/jama.2020.21566

41. Park CH, Valore E V., Waring AJ, Ganz T. Hepcidin, a Urinary Antimicrobial Peptide Synthesized in the Liver. *J Biol Chem*. 2001;276(11):7806-7810. doi:10.1074/jbc.M008922200

42. Nairz M, Metzendorf C, Vujic-Spasic M, et al. Cell-specific expression of Hfe determines the outcome of Salmonella enterica serovar Typhimurium infection in mice. *Haematologica*. 2021;106(12):3149-3161. doi:10.3324/haematol.2019.241745

43. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet*. 1996;13(4):399-408. doi:10.1038/ng0896-399

44. Nedwin GE, Naylor SL, Sakaguchi AY, et al. Human lymphotoxin and tumor necrosis factor genes: Structure, homology and chromosomal localization. *Nucleic Acids Res*. 1985;13(17):6361-6373. doi:10.1093/nar/13.17.6361

45. Dunham I, Sargent CA, Trowsdalet J, Campbell RD. Molecular mapping of the human major histocompatibility complex by pulsed-field gel electrophoresis (HLA/gene map/tumor necrosis factor/cosmid cloning). *Immunology*. 1987;84(October):7237-7241.

	Cohort		
	Copenhagen City Heart Study	Copenhagen General Population Study	Danish General Suburban Population Study
Individuals, no.	13,661	108,061	20,466
Plasma iron, no. (%)	8,884 (65) *	107,343 (99)	20,429 (100)
Plasma transferrin saturation, no. (%)	8,871 (65) *	107,302 (99)	20,426 (100)
Plasma ferritin, no. (%)	8,999 (66) *	8,632 (8)	20,389 (100)
Haemochromatosis genotyped, no. (%)	9,174 (67) *	103,276 (96)	20,092 (98)
Age, years (IQR)	61 (49-70)	58 (48-67)	57 (46-67)
Year of birth (IQR)	1928 (1919-1940)	1951 (1942-1961)	1954 (1945-1966)
Men, no. (%)	6,438 (47)	48,640 (45)	9,344 (46)
Pre-menopausal women, no. (% of women)	2,527 (35)	19,298 (32)	3,962 (36)
Ever smokers, no. (%)	10,817 (79)	62,463 (58)	11,470 (56)
Cumulative smoking, pack-years (IQR) †	22 (9-38)	16 (6-30)	18 (8-31)
Alcohol consumption >168/84g/week, no. (%) ‡	3,742 (27)	42,232 (39)	4,852 (24)
Body mass index, kg/m^2 (IQR)	24.9 (22.5-27.8)	25.6 (23.2-28.4)	26.1 (23.5-29.2)
Any comorbidity, no. (%) §	1,570 (11)	23,209 (21)	4,355 (21)
Plasma C-reactive protein, mg/l (IQR)	1.7 (1.3-3.0)	1.4 (0.9-2.3)	1.4 (0.7-3.0)

Table 1: Baseline characteristics of 142,188 individuals from the general population according to study cohort.

no. (%) is displayed for categorical variables while median (interquartile range) is displayed for continuous variables.

no.: number. IQR: interquartile range.

* In the Copenhagen City Heart study, 8,999 individuals were included from the 1981-83 examination and 9,725 individuals were included from the 1991-94 examination, of whom 5,063 individuals attended both examinations. Only individuals who attended the 1991-1994 examination had iron (8,884 individuals out of 10,135 attending, 88%), transferrin saturation (8,871 individuals out of 10,135 attending, 88%), and haemochromatosis genotype (9,174 individuals out of 10,135 attending, 91%) measured. Only individuals who attended the 1981-1983 examination had ferritin measured (8,999 individuals out of 12,698 attending, 71%).

† Ever smokers only

‡ >168 g/week for men and >84g/week for women

§ Any comorbidity as defined by the Charlson comorbidity index

Legends for figures

Figure 1

Kaplan-Meier curves for risk of any infection according to plasma iron, transferrin saturation, ferritin, and *HFE* genotype. The number of individuals at risk and number of infections shown are for all individuals that have measurements of iron, transferrin saturation, ferritin, or *HFE* genotype, respectively. The graphical curves depict solely the 0^{th}-5^{th} percentile, 26^{th}-74^{th} percentile, and 95^{th}-100^{th} percentile when studying risk of infection according to iron, transferrin saturation, or ferritin, while the curves depict solely C282Y/C282Y and non-carrier/non-carrier individuals when studying risk of infection according to *HFE* genotype. Statistical power is limited in individuals younger than 40 years of age as a result of relatively few individuals enrolled before age 40. Therefore, curves are shown from age 40 years and onwards to avoid single infection events in younger individuals leading to large fluctuations in the graphical depiction of Kaplan-Meier estimates due to low statistical power. P-values were calculated using a log-rank test.

no.: number.

ref.: reference

Figure 2

Risk of any infection according to levels of plasma iron, transferrin saturation, and ferritin. Solid red lines are hazard ratios obtained using Cox regression, broken black lines indicate 95%CIs. Analyses were multivariable adjusted for age, year of birth, sex, smoking status, cumulative smoking in pack-years, alcohol consumption, body mass index, menopausal status, study cohort, Charlson comorbidity index, and level of C-reactive protein. Reference for the continuous models (splines) is the median value of plasma iron (14 μmol/l), transferrin saturation (23%), or ferritin (100 μg/l) in the respective analyses. Total number of individuals in the analyses on risk of any infection according to iron, transferrin saturation, and ferritin were 136,656, 136,599, and 38,020, respectively. All individuals who had a measurement of iron, transferrin saturation, or ferritin were included in the categorical analyses and in modelling of the splines, but when drawing the spline curves the X-axis was capped at the 99.9^{th} percentile.

* Hazard ratio, 95% CI, and P-value obtained using Cox regression.

† Subhazard ratio and 95% CI obtained using Fine-Gray competing risk regression on risk of any infection with death from any cause as competing risk

CI: confidence interval, ref.: reference group, sat.: saturation, no.: number

Figure 3

Change in plasma iron and transferrin saturation for each individual between first blood sample drawn at study enrolment and second blood sample drawn for a repeat measurement of plasma iron or transferrin saturation. For a total of 32,141 individuals, a second blood sample for a repeat measurement of iron and/or transferrin saturation was obtained at a median of 10 years (IQR: 9.6-10.7 years, range: 0.7-15.8 years) after the first blood sample. Median modulus value (non-negative value) for change in plasma iron was 3.2 µmol/l (IQR: 1.7-5.9). For transferrin saturation median modulus value (non-negative value) for change was 6% (IQR: 3-10%). Importantly, all main analyses on risk of infections were based on the blood samples drawn at the day of study enrolment (first blood sample), except for Supplementary Figure S4 where the second blood sample for repeat measurements of iron and transferrin saturation was used to model risk of infection. No individuals had repeat measurements of ferritin. no.: number, IQR: interquartile range.

Figure 4

Risk of pneumonia and sepsis according to levels of plasma iron, transferrin saturation, and ferritin. Solid red lines are hazard ratios, broken black lines indicate 95%CIs. Analyses were multivariable adjusted for age, year of birth, sex, smoking status, cumulative smoking in pack-years, alcohol consumption, body mass index, menopausal status, study cohort, Charlson comorbidity index, and level of C-reactive protein. Reference for the continuous models (splines) is the median value of plasma iron (14 µmol/l), transferrin saturation (23%), or ferritin (100 µg/l) in the respective analyses. All individuals who had a measurement of iron, transferrin saturation, or ferritin were included in the categorical analyses and in modelling of the splines, but when drawing the spline curves the X-axis was capped at the 99.9th percentile. CI: confidence interval, ref.: reference, no.: number.

Figure 5

Risk of any infection, pneumonia, sepsis, and death from infectious disease according to haemochromatosis genotypes C282Y and H63D.

Δiron, Δtransferrin saturation, and Δferritin indicate the mean difference as percentage of iron, transferrin saturation, and ferritin, respectively compared to non-carrier/non-carrier individuals.

Dots indicate hazard ratio; solid vertical lines indicate 95% confidence interval.

Non-carrier/non-carrier: Non-carrier for both C282Y and H63D. H63D/non-carrier: Heterozygous for the H63D variant. H63D/H63D: Homozygous for the H63D variant. C282Y/non-carrier: Heterozygous for the C282Y variant. C282Y/H63D: Compound heterozygous for the C282Y and H63D variants. C282Y/C282Y: Homozygous for the C282Y variant.

CI: confidence interval, no.: number

Figure 6

Risk of any infection for C282Y homozygotes (C282Y/C282Y) compared to individuals non-carrier for both C282Y and H63D (non-carrier/non-carrier) stratified by levels of plasma iron, transferrin saturation, and ferritin at study enrolment.

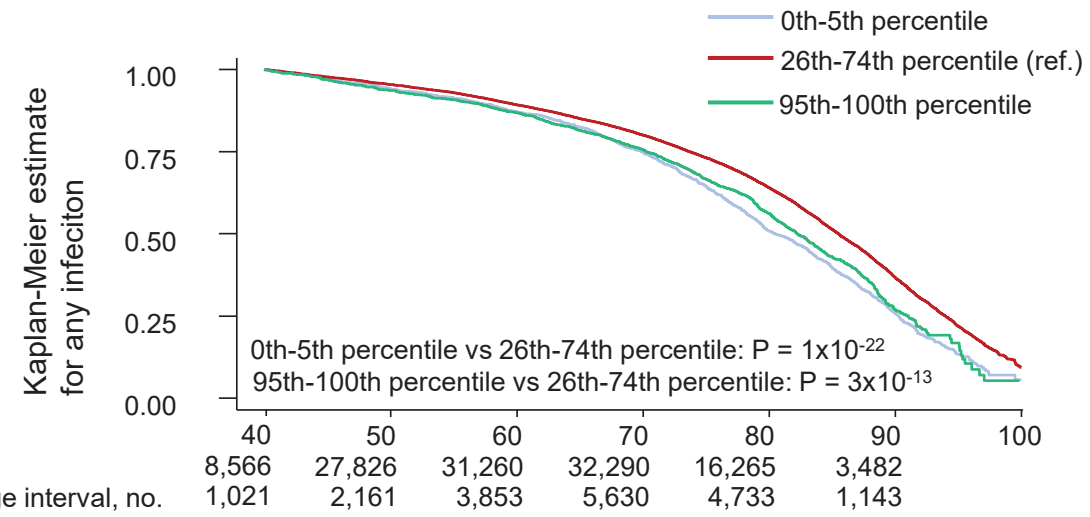
Normal iron was defined as iron from 9-34 µmol/l and high iron defined as iron >34 µmol/l. Normal transferrin saturation was defined as transferrin saturation from 10-45% for women under 50 years of age and 15-45% for women above 50 years of age and men regardless of age. High transferrin saturation was defined as transferrin saturation > 45% regardless of sex and age. Normal ferritin was defined as ferritin from 12-200 µg/l for women and 12-300µg/l for men. High ferritin was defined as ferritin > 200 µg/l for women and >300 for men.

Dots indicate hazard ratio; solid vertical lines indicate 95% confidence interval.

CI: confidence interval, no.: number, sat.: saturation

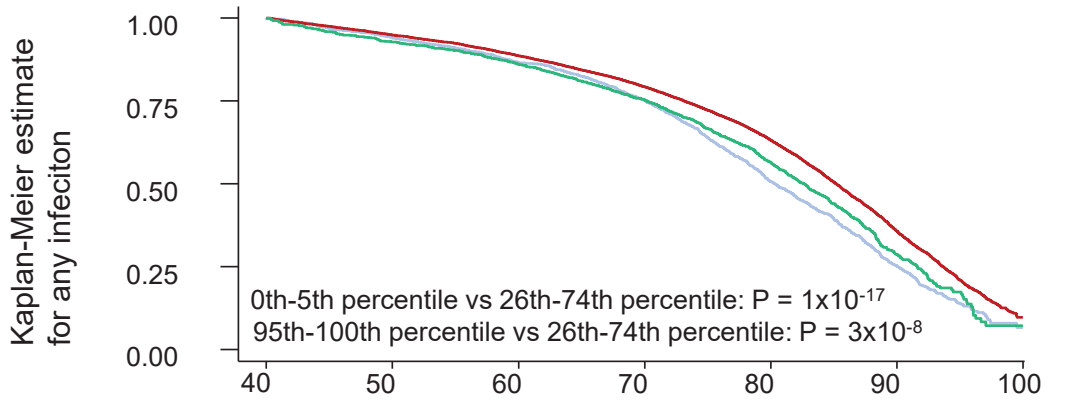
Figure 1

Iron



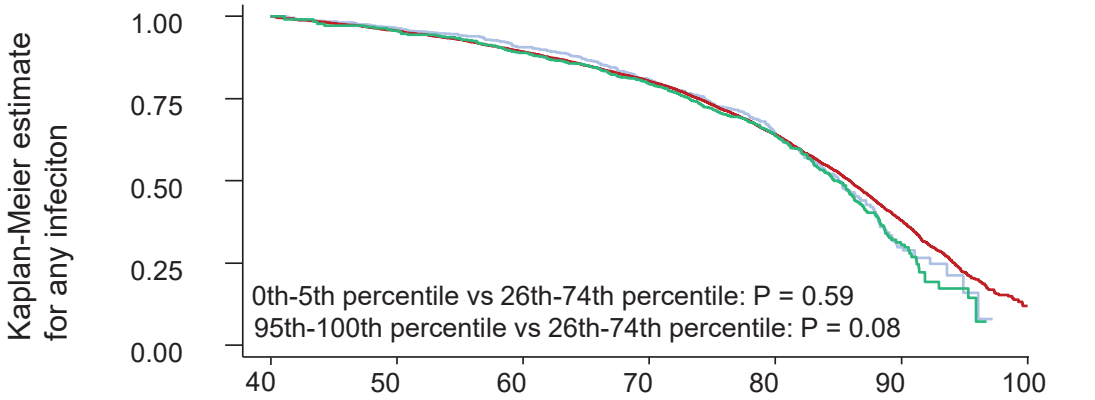
Age (years)	40	50	60	70	80	90	100
Individuals at risk, no.	8,566	27,826	31,260	32,290	16,265	3,482	
Infections during 10 year age interval, no.	1,021	2,161	3,853	5,630	4,733	1,143	

Transferrin saturation



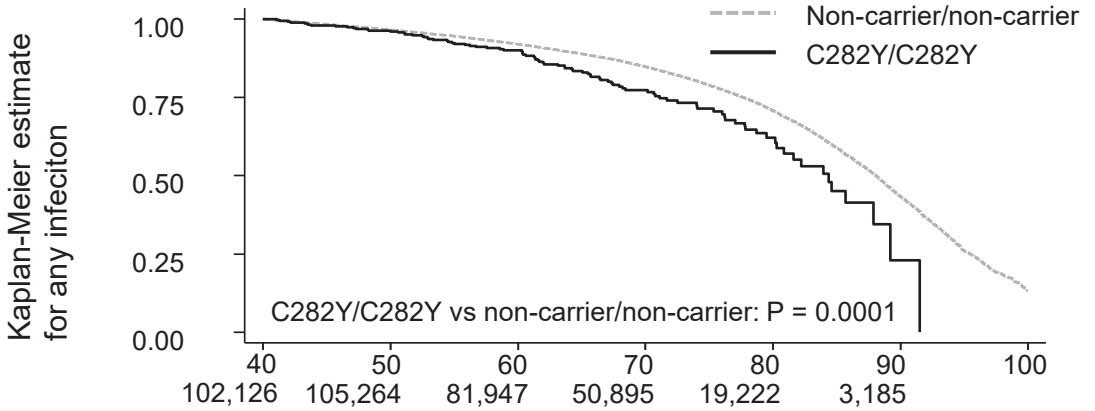
Age (years)	40	50	60	70	80	90	100
Individuals at risk, no.	8,562	27,819	31,245	32,278	16,258	3,480	
Infections during 10 year age interval, no.	1,021	2,161	3,848	5,627	4,732	1,142	

Ferritin



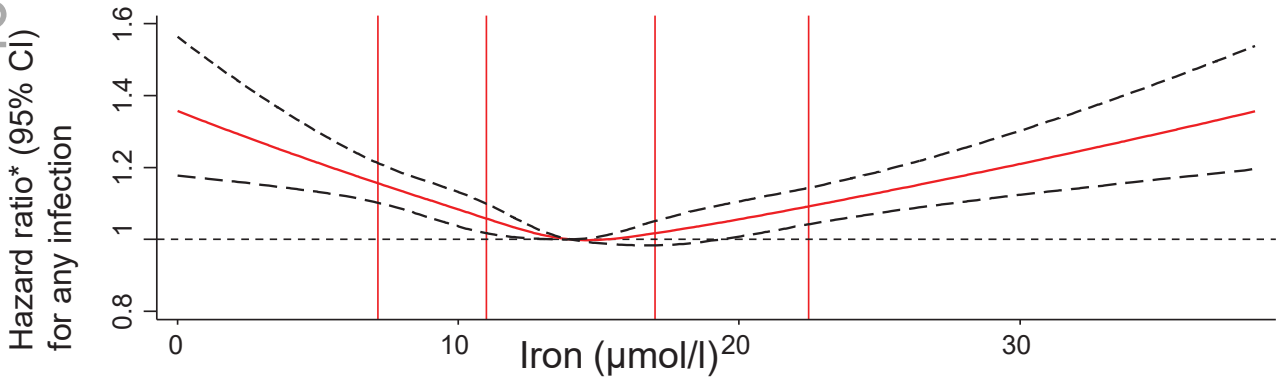
Age (years)	40	50	60	70	80	90	100
Individuals at risk, no.	3,278	8,007	10,908	11,409	6,160	1,284	
Infections during 10 year age interval, no.	259	669	1,264	1,997	1,706	422	

HFE genotype

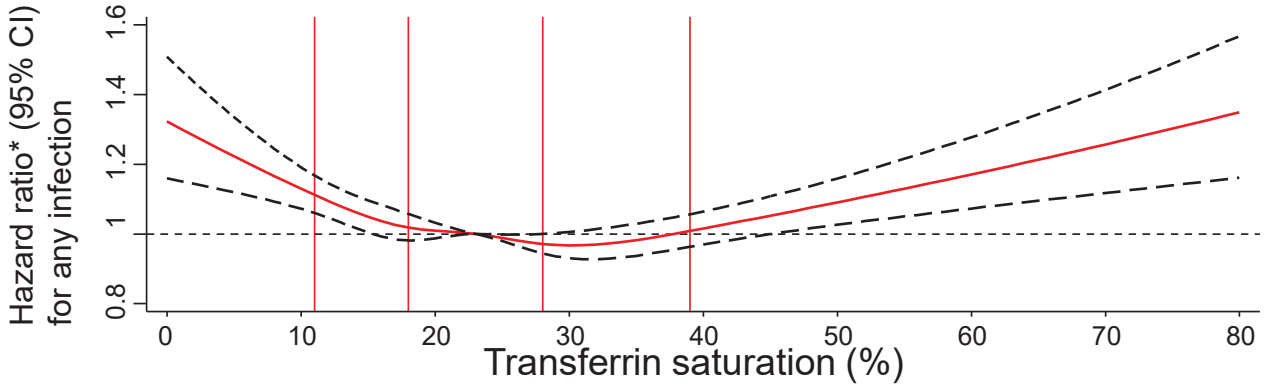


Age (years)	40	50	60	70	80	90	100
Individuals at risk, no.	102,126	105,264	81,947	50,895	19,222	3,185	
Infections during 10 year age interval, no.	3,701	4,623	5,337	5,761	4,163	959	

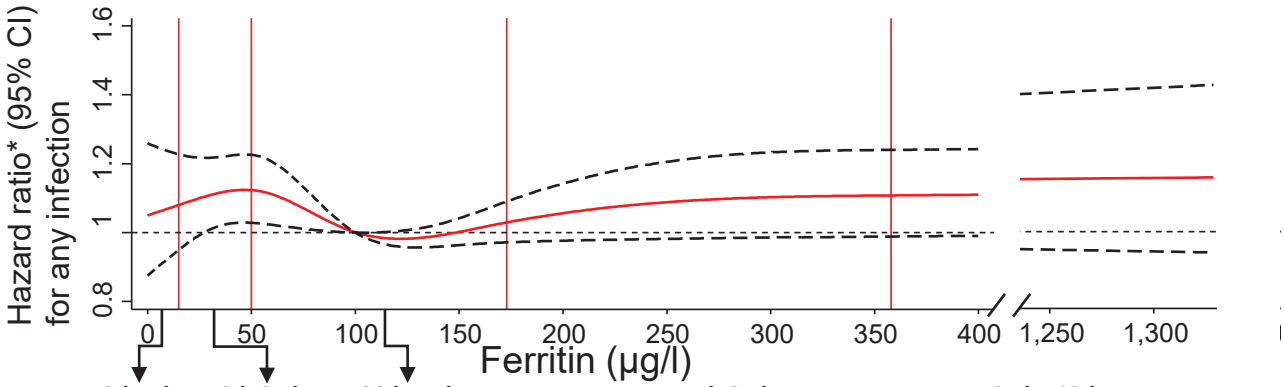
Figure 2



Percentiles	0th-5th	6th-25th	26th-74th	75th-94th	95th-100th
Iron (µmol/l)	0-7.1	7.2-11.0	11.1-17.0	17.1-22.4	22.5-130.2
Individuals, no.	7,234	29,815	63,570	28,859	7,178
Infections, no.	1,111	4,338	8,409	4,015	1,037
Hazard ratio*	1.20	1.08	(ref.)	1.04	1.14
(95% CI)	(1.12-1.28)	(1.04-1.12)	(ref.)	(1.00-1.08)	(1.07-1.22)
P	$4x10^{-8}$	0.0001	(ref.)	0.07	$9x10^{-5}$
When additionally performing competing risk analysis with death as competing risk:					
Subhazard ratio†	*1.15*	*1.07*	*(ref.)*	*1.02*	*1.10*
(95% CI)	*(1.08-1.23)*	*(1.03-1.11)*	*(ref.)*	*(0.98-1.06)*	*(1.03-1.17)*



Percentiles	0th-5th	6th-25th	26th-74th	75th-94th	95th-100th
Transferrin sat. (%)	0-11.0	11.1-18.0	18.1-27.9	28.0-38.9	39.0-195
Individuals, no.	7,401	31,749	58,997	32,176	6,276
Infections, no.	1,068	4,362	8,027	4,450	993
Hazard ratio*	1.18	1.03	(ref.)	0.98	1.10
(95% CI)	(1.10-1.26)	(1.00-1.07)	(ref.)	(0.95-1.02)	(1.03-1.17)
P	$9x10^{-7}$	0.09	(ref.)	0.33	0.007
When additionally performing competing risk analysis with death as competing risk:					
Subhazard ratio†	*1.14*	*1.02*	*(ref.)*	*0.98*	*1.07*
(95% CI)	*(1.07-1.22)*	*(0.98-1.06)*	*(ref.)*	*(0.95-1.02)*	*(1.00-1.14)*



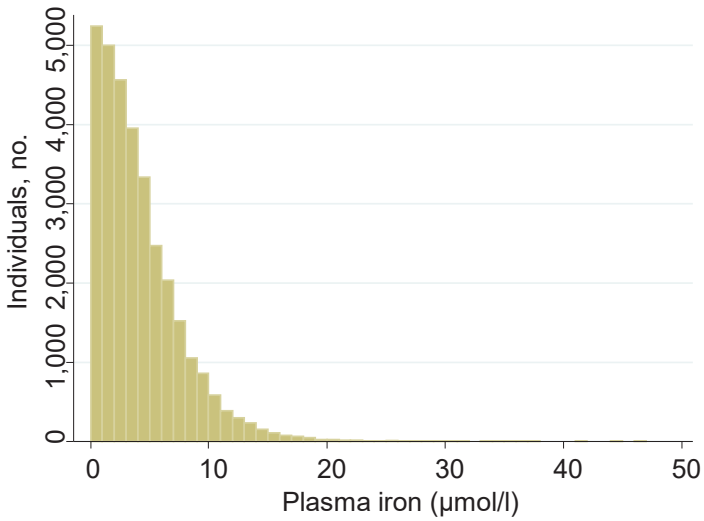
Percentiles	0th-5th	6th-25th	26th-74th	75th-94th	95th-100th
Ferritin (µg/l)	0-15.0	15.1-50.0	50.1-172.9	173.0-357.9	358.0-17200
Individuals, no.	2,095	7,427	18,812	7,775	1,911
Infections, no.	278	1,161	3,297	1,371	318
Hazard ratio*	1.06	1.07	(ref.)	1.03	1.03
(95% CI)	(0.93-1.21)	(1.00-1.15)	(ref.)	(0.96-1.09)	(0.91-1.16)
P	0.36	0.04	(ref.)	0.44	0.65
When additionally performing competing risk analysis with death as competing risk:					
Subhazard ratio†	*1.00*	*1.07*	*(ref.)*	*1.01*	*0.91*
(95% CI)	*(0.88-1.13)*	*(1.00-1.15)*	*(ref.)*	*(0.95-1.08)*	*(0.81-1.02)*

Figure 3

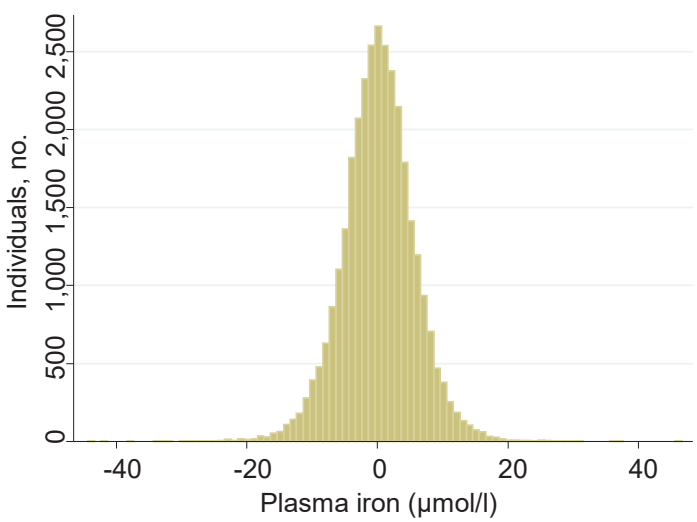
Iron

Individuals with second blood sample drawn for a repeat measurement of plasma iron: 32,141

Modulus value (non-negative value) for change in iron from first to second blood sample



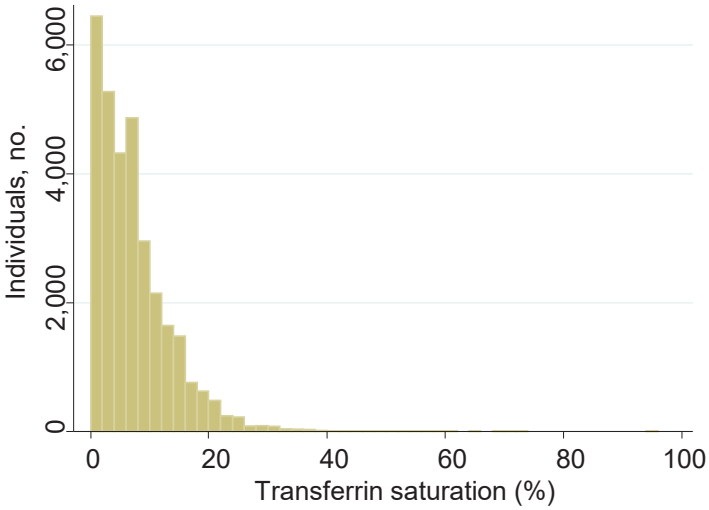
Change in iron from first to second blood sample



Transferrin saturation

Individuals with second blood sample drawn for a repeat measurement of transferrin saturation: 31,942

Modulus value (non-negative value) for change in transferrin saturation from first to second blood sample



Change in transferrin saturation from first to second blood sample

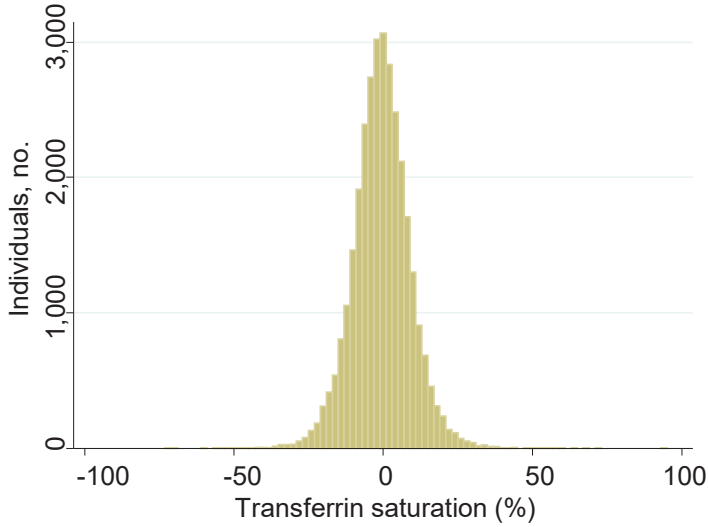
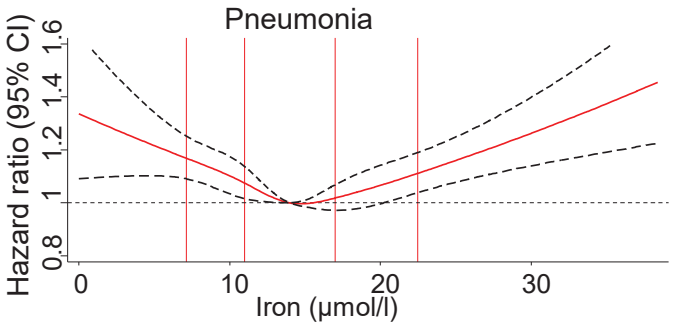
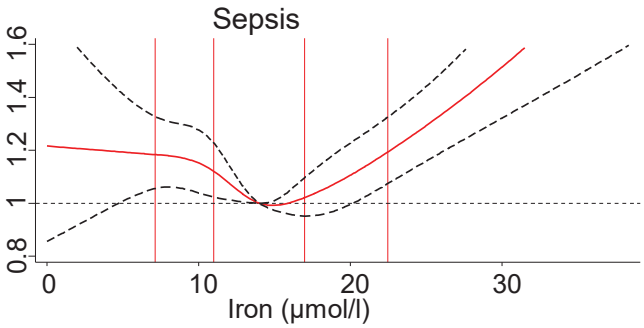


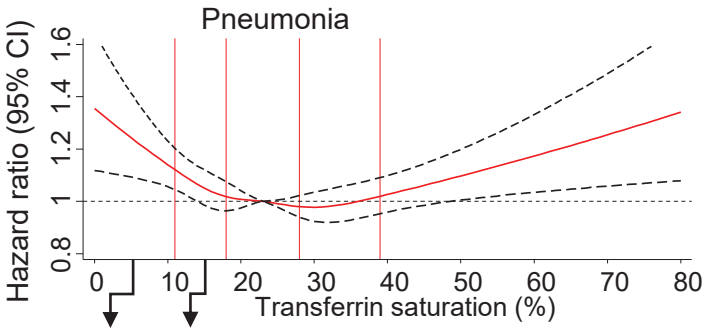
Figure 4

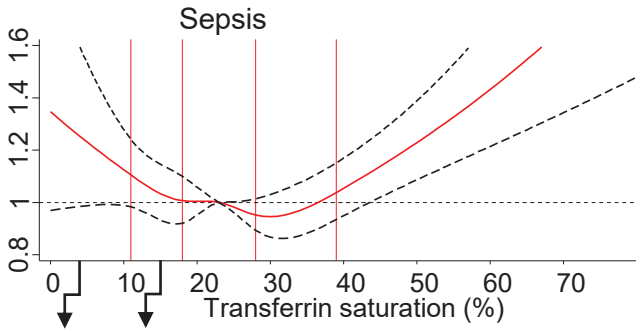




Percentiles	0th-5th	6th-25th	26th-74th	75th-94th	95th-100th
Individuals, no.	7,234	29,815	63,570	28,859	7,178
Infections, no.	561	2,047	3,948	1,820	514
Hazard ratio	1.20	1.07	(ref.)	1.01	1.22
(95% CI)	(1.10-1.32)	(1.01-1.13)	(ref.)	(0.96-1.07)	(1.11-1.34)
P	$8x10^{-5}$	0.02	(ref.)	0.61	$2x10^{-5}$

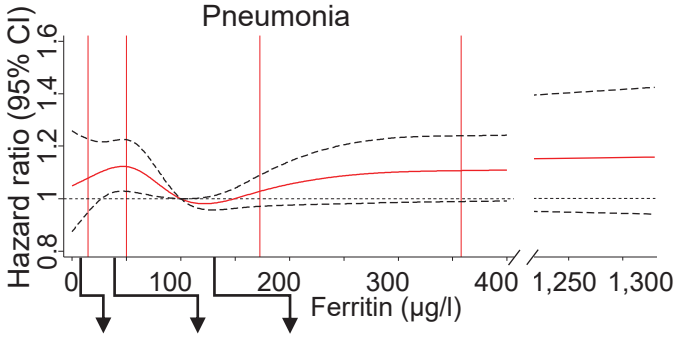
Percentiles	0th-5th	6th-25th	26th-74th	75th-94th	95th-100th
Individuals, no.	7,234	29,815	63,570	28,859	7,178
Infections, no.	186	771	1,532	795	246
Hazard ratio	1.18	1.10	(ref.)	1.06	1.38
(95% CI)	(1.01-1.38)	(1.01-1.21)	(ref.)	(0.98-1.16)	(1.20-1.58)
P	0.04	0.03	(ref.)	0.15	$4x10^{-6}$

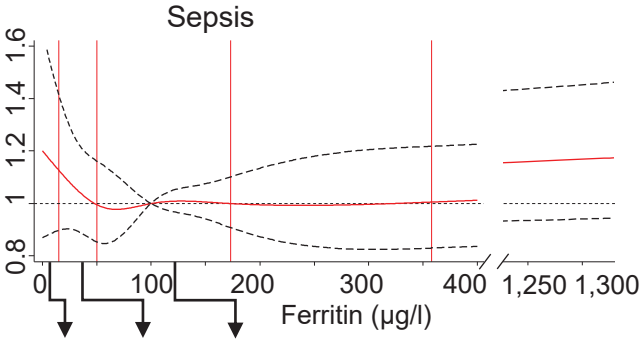




Percentiles	0th-5th	6th-25th	26th-74th	75th-94th	95th-100th
Individuals, no.	7,401	31,749	58,997	32,176	6,276
Infections, no.	518	2,028	3,759	2,100	480
Hazard ratio	1.20	1.02	(ref.)	0.98	1.11
(95% CI)	(1.09-1.32)	(0.97-1.08)	(ref.)	(0.93-1.04)	(1.01-1.22)
P	0.0002	0.50	(ref.)	0.56	0.04

Percentiles	0th-5th	6th-25th	26th-74th	75th-94th	95th-100th
Individuals, no.	7,401	31,749	58,997	32,176	6,276
Infections, no.	162	742	1,504	883	239
Hazard ratio	1.12	1.01	(ref.)	0.97	1.27
(95% CI)	(0.95-1.32)	(0.92-1.10)	(ref.)	(0.89-1.06)	(1.11-1.46)
P	0.20	0.84	(ref.)	0.51	0.0007





Percentiles	0th-5th	6th-25th	26th-74th	75th-94th	95th-100th
Individuals, no.	2,095	7,427	18,812	7,775	1,911
Infections, no.	140	610	1,800	748	172
Hazard ratio	1.10	1.06	(ref.)	1.03	1.05
(95% CI)	(0.92-1.31)	(0.96-1.16)	(ref.)	(0.94-1.12)	(0.90-1.23)
P	0.32	0.25	(ref.)	0.52	0.55

Percentiles	0th-5th	6th-25th	26th-74th	75th-94th	95th-100th
Individuals, no.	2,095	7,427	18,812	7,775	1,911
Infections, no.	52	169	586	257	72
Hazard ratio	1.33	0.97	(ref.)	0.99	1.19
(95% CI)	(0.98-1.79)	(0.81-1.16)	(ref.)	(0.85-1.15)	(0.92-1.52)
P	0.06	0.73	(ref.)	0.90	0.18

Figure 5

Any infection

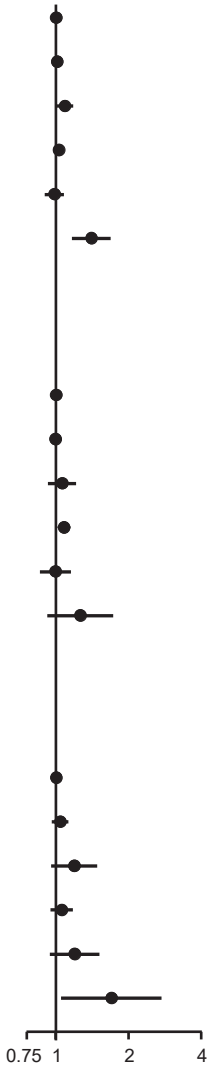
	ΔIron	ΔTransferrin saturation	ΔFerritin	Individuals, no.	Infections, no.	Age and sex adjusted hazard ratio (95% CI)	P
Non-carrier/non-carrier	0% (ref.)	0% (ref.)	0% (ref.)	87,313	19,663	1.00 (reference)	
H63D/non-carrier	8%	11%	8%	27,820	6,269	1.01 (0.98 to 1.04)	0.52
H63D/H63D	22%	31%	23%	2,358	573	1.09 (1.00 to 1.18)	0.05
C282Y/non-carrier	11%	18%	11%	12,514	2,854	1.03 (0.99 to 1.07)	0.19
C282Y/H63D	35%	55%	39%	2,115	464	0.98 (0.90 to 1.08)	0.73
C282Y/C282Y	84%	159%	316%	422	113	1.40 (1.16 to 1.68)	0.0004

Pneumonia

Non-carrier/non-carrier	0% (ref.)	0% (ref.)	0% (ref.)	87,313	7,737	1.00 (reference)	
H63D/non-carrier	8%	11%	8%	27,820	2,411	0.99 (0.95 to 1.04)	0.81
H63D/H63D	22%	31%	23%	2,358	222	1.06 (0.93 to 1.21)	0.40
C282Y/non-carrier	11%	18%	11%	12,514	1,171	1.08 (1.01 to 1.15)	0.02
C282Y/H63D	35%	55%	39%	2,115	181	0.99 (0.86 to 1.15)	0.94
C282Y/C282Y	84%	159%	316%	422	39	1.26 (0.92 to 1.72)	0.15

Sepsis

Non-carrier/non-carrier	0% (ref.)	0% (ref.)	0% (ref.)	87,313	2,557	1.00 (reference)	
H63D/non-carrier	8%	11%	8%	27,820	825	1.04 (0.96 to 1.12)	0.33
H63D/H63D	22%	31%	23%	2,358	82	1.19 (0.95 to 1.48)	0.12
C282Y/non-carrier	11%	18%	11%	12,514	380	1.05 (0.95 to 1.17)	0.34
C282Y/H63D	35%	55%	39%	2,115	71	1.19 (0.94 to 1.51)	0.14
C282Y/C282Y	84%	159%	316%	422	17	1.69 (1.05 to 2.73)	0.03



Death from infectious disease

	ΔIron	ΔTransferrin saturation	ΔFerritin	Individuals, no.	Infectious disease deaths, no.	Age and sex adjusted hazard ratio (95% CI) for death from infectious disease	P
Non-carrier/non-carrier	0% (ref.)	0% (ref.)	0% (ref.)	87,313	2,017	1.00 (reference)	
H63D/non-carrier	8%	11%	8%	27,820	624	1.01 (0.93 to 1.11)	0.77
H63D/H63D	22%	31%	23%	2,358	60	1.13 (0.87 to 1.46)	0.36
C282Y/non-carrier	11%	18%	11%	12,514	299	1.09 (0.97 to 1.23)	0.16
C282Y/H63D	35%	55%	39%	2,115	47	0.99 (0.74 to 1.32)	0.96
C282Y/C282Y	84%	159%	316%	422	15	2.34 (1.41 to 3.90)	0.001

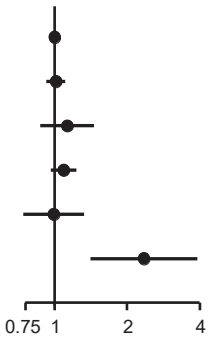
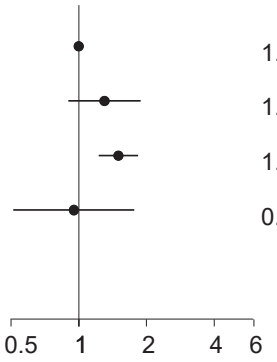


Figure 6

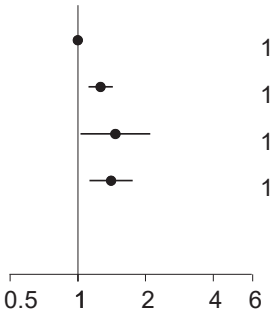
Iron

	Individuals, no.	Infections, no.	Age and sex adjusted hazard ratio for any infection (95% CI)	P
Non-carrier/non-carrier *with normal iron*	75,725	16,880	1.00 (reference)	
Non-carrier/non-carrier *with high iron*	114	28	1.30 (0.90 to 1.88)	0.17
C282Y/C282Y *with normal iron*	348	97	1.50 (1.23 to 1.83)	$7x10^{-5}$
C282Y/C282Y *with high iron*	56	10	0.95 (0.51 to 1.76)	0.86



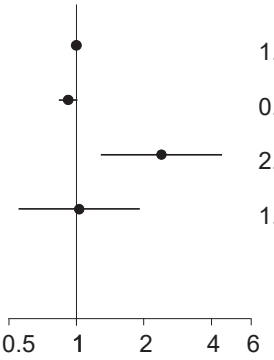
Transferrin saturation

	Individuals, no.	Infections, no.	Age and sex adjusted hazard ratio for any infection (95% CI)	P
Non-carrier/non-carrier *with normal transferrin sat.*	75,646	16,616	1.00 (reference)	
Non-carrier/non-carrier *with high transferrin sat.*	893	253	1.26 (1.12 to 1.43)	0.0002
C282Y/C282Y *with normal transferrin sat.*	115	30	1.47 (1.03 to 2.10)	0.04
C282Y/C282Y *with high transferrin sat.*	294	78	1.41 (1.13 to 1.76)	0.003



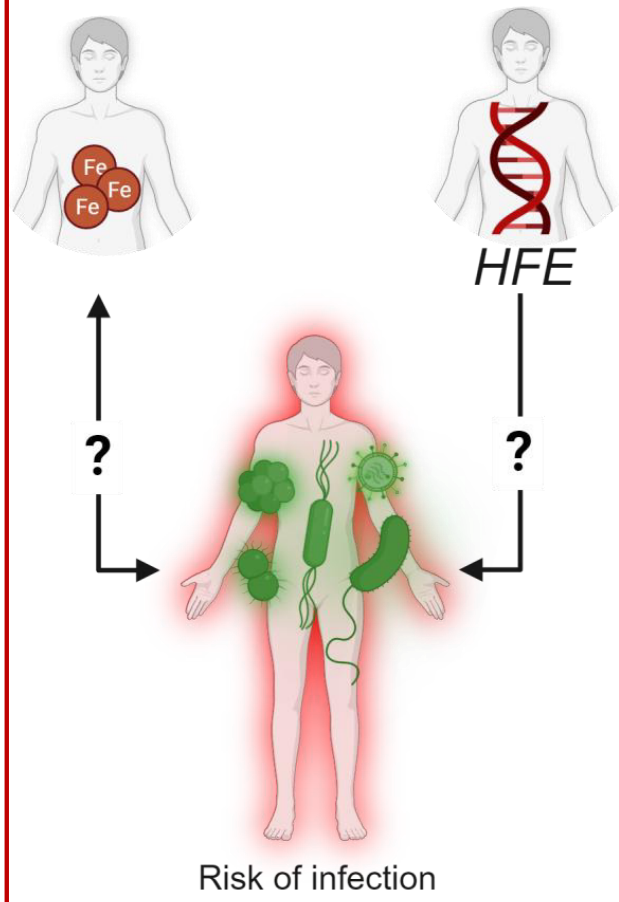
Ferritin

	Individuals, no.	Infections, no.	Age and sex adjusted hazard ratio for any infection (95% CI)	P
Non-carrier/non-carrier *with normal ferritin*	19,355	4,391	1.00 (reference)	
Non-carrier/non-carrier *with high ferritin*	2,116	476	0.92 (0.84 to 1.01)	0.09
C282Y/C282Y *with normal ferritin*	28	10	2.39 (1.29 to 4.45)	0.006
C282Y/C282Y *with high ferritin*	53	10	1.03 (0.55 to 1.91)	0.93



Iron, haemochromatosis genotypes, and risk of infections: a cohort study of 142,188 general population individuals

Research question



Methods

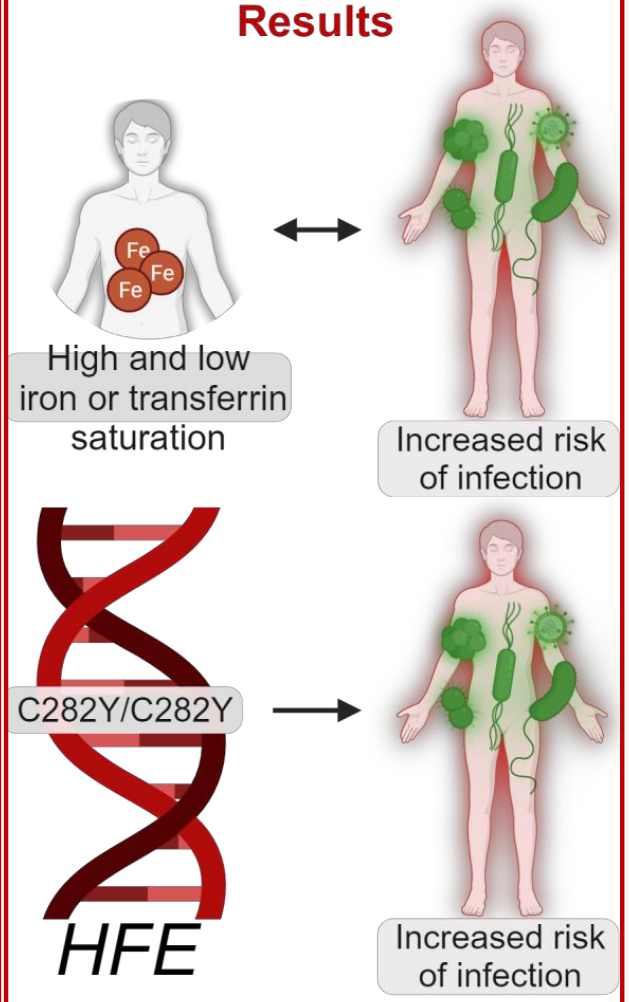
Study population
- 142,188 general population individuals

Measured
- Plasma iron
- Transferrin saturation
- Ferritin
- *HFE* genotype (H63D & C282Y)

Follow-up
- Median 8 years after study enrolment

Information on deaths and hospilizations due to infections obtained from national Danish registries

Results



Conclusion: Haemochromatosis C282Y homozygotes had increased risk of any infection, and markedly increased risk of sepsis and death from infections. C282Y homozygotes with normal iron, transferrin saturation, or ferritin, not currently recommended for genotyping, had high infection risk. *[Reference to article]*

Created with BioRender.com