Morbidity, risk of cancer and mortality in 3645 HFE mutations carriers

Hannes Hagström1,2,3 | Nelson Ndegwa4,5 | Molly Jalmeus3 | Mattias Ekstedt6 | Iris Posserud7 | Fredrik Rorsman8 | Nils Nyhlin9 | Daniel Klintman10 | Mårten Werner11 | Hanns-Ulrich Marschall12 | Johan Askling2 | Per Stål1,3 | the Swedish Hepatology Study Group (SweHep)

1Division of Hepatology, Department of Upper GI diseases, Karolinska University Hospital, Stockholm, Sweden
2Clinical Epidemiology Division, Department of Medicine, Solna, Karolinska Institutet, Stockholm, Sweden
3Department of Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden
4Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
5Division of Surgery, Department of Clinical Science Intervention and Technology, Karolinska Institutet, and Oesophageal and Gastric Cancer Unit, Karolinska University Hospital, Stockholm, Sweden
6Division of Diagnostics and Specialist Medicine, Department of Health, Medicine and Caring Sciences, Linköping University, Linköping, Sweden
7Department of Internal Medicine and Clinical Nutrition, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
8Department of Gastroenterology and Hepatology, Uppsala University Hospital, Uppsala, Sweden
9Department of Gastroenterology, Faculty of Medicine and Health, Örebro University, Örebro, Sweden
10Department of Gastroenterology and Hepatology, Skåne University Hospital, Malmö, Sweden
11Department of Public Health and Clinical Medicine, Medicine, Umeå University, Umeå, Sweden
12Department of Molecular and Clinical Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Correspondence
Hannes Hagström, C1:77, Division of Hepatology, Department of Upper GI Diseases, Karolinska University Hospital, 141 86 Stockholm, Sweden. Email: hannes.hagstrom@ki.se

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Abstract

Background & Aims: Mutations in the HFE gene can lead to hereditary haemochromatosis (HH) and have been suggested to increase the risk of extra-hepatic diseases, especially breast and colorectal cancer. Here we investigated long-term outcomes of Swedish patients with HFE mutations.

Methods: We identified 3645 patients with a homozygous p.C282Y (62%) or a compound heterozygous p.C282Y/p.H63D (38%) mutation from eight centres in Sweden between 1997 and 2017. These were matched 1:10 by age, sex and county of residence to reference individuals from the general population. We ascertained incident outcomes until the end of 2017 by linkage to national registers. Studied outcomes were HH, cirrhosis, hepatocellular carcinoma (HCC), breast cancer (in women), colorectal cancer, type 1 and 2 diabetes, hypothyroidism, Parkinson’s disease and mortality. Cox proportional hazards regression was used to estimate hazard ratios for these outcomes.

Results: Median age at diagnosis was 52 years, 44% were females. During a mean follow-up of 7.9 years, we found an increased risk for HCC, HH, cirrhosis, type 2
diabetes, osteoarthritis and death. Excess mortality was only seen in men. No increased risk was seen for colorectal or breast cancer. Liver-related outcomes were rare, with a cumulative incidence of <1%.

Conclusions: Individuals found to be HFE mutation carriers in a university hospital setting had an increased risk for mortality in men, along with increased risks of cirrhosis, HCC, diabetes type 2, and osteoarthritis. In general, the absolute risk for adverse outcomes was low and no increased risk for colon or breast cancer was observed.

KEYWORDS
C282Y, epidemiology, H63D, hemochromatosis, prognosis

1 INTRODUCTION

Mutations in the HFE gene are linked to hereditary haemochromatosis (HH) type 1, which is the most common genetic disease in individuals of European descent. The two most important HFE mutations are p.Cys282Tyr (p.C282Y) and p.His63Asp (p.H63D), found in the heterozygous form in 4%-12% and 10%-18% respectively, in the European population. HFE encodes a cell surface protein that regulates hepcidin expression and iron absorption. Homozygosity for p.C282Y results in disrupted HFE signalling, hepcidin deficiency and increased iron release from enterocytes and macrophages, which may lead to the HH phenotype. Persons that are compound heterozygous for p.C282Y and p.H63D typically have a milder phenotype. A homozygous p.C282Y mutation in HFE is found in 80%-90% of patients with HH type 1, whereas only about 5% are compound heterozygotes. However, the homozygous p.C282Y genotype is present in approximately 0.5% of the general population with European descent, and only a minority of these develop phenotypic HH. In different population studies, the penetrance of clinical HH in p.C282Y homozygotes varies from 1% to 24%, with the highest numbers in men.

Previous cohort studies have linked HH to an increased risk for liver cirrhosis and hepatocellular carcinoma (HCC), osteoarthritis, diabetes mellitus and extrahepatic malignancies, mainly colorectal and breast cancer. After the introduction of HFE genotyping in 1996, similar cohort studies have been performed on HFE mutation carriers with or without clinical HH, but the results regarding the risk of extrahepatic malignancies are conflicting, which could possibly be attributed to differences in why the included populations were sampled for HFE (e.g. asymptomatic blood donors vs. targeted testing in symptomatic individuals). Associations of HFE mutations have also been made with non-malignant diseases such as type 2 diabetes (T2D), Parkinson’s disease and osteoarthritis.

However, most previous investigations on especially cancer risk in HH carriers have been conducted in case-control studies, and studies of long-term outcomes associated with mutations in HFE are scarce. Recently, a large population-based study presented data from the UK biobank. The authors found an increased risk for incident liver cirrhosis, HCC, diabetes mellitus and osteoarthritis in a large cohort of individuals homozygous for the p.C282Y mutation during a mean follow-up of 7 years. However, the risk of overall mortality was not increased compared to other participants in the UK biobank, and the risk of extra-hepatic malignancies was not reported.

In the present study we aimed to examine long-term outcomes in a large cohort of individuals with homozygous or compound heterozygous HFE mutations in Sweden and compare disease risk to reference individuals from the general population.

2 MATERIAL AND METHODS

This was a multi-centre cohort study with retrospectively assembled data through the Swedish hepatology network (SweHep, www.swehep.se), consisting of clinical researchers from all eight Swedish university hospitals. In 2018, we contacted all laboratory departments reporting to these hospitals and obtained data on all persons who had been tested for HFE mutations and found to have a homozygous HFE p.C282Y mutation or a compound heterozygote mutation in p.C282Y and p.H63D between 1 January 1997 and 31 December 2017. No information on why the HFE-test was performed was available, and we did not obtain data on those that tested negatively for HFE. Study baseline was defined as the date of HFE testing.

2.1 Variables

We recorded the following variables at baseline: Age, sex, type of HFE mutation (homozygous for p.C282Y or compound heterozygote for both p.C282Y and p.H63D), ferritin (measured in μg/L) and alanine aminotransferase (ALT, measured in μkat/L). Data on ALT and ferritin was not automatically reported by the laboratories but was instead recorded from medical charts separately by one of the co-authors where available within 1 year before HFE testing. We did not record ALT and ferritin obtained after HFE diagnosis, as initiated treatment (phlebotomy) might have impacted such results.
2.2 Follow-up

For each person with a HFE mutation, up to 10 reference individuals were obtained from the general population as recorded in the Total Population Register. Reference individuals were matched on sex, age and county of residence at the time of HFE testing. Data on HFE genotype, ALT and ferritin were not available in the reference population. We excluded any person missing a Swedish personal identity number (six persons with HFE mutations and no reference individuals) or who had undergone a liver transplantation before baseline (four persons with HFE mutations and five reference individuals). Removal of a person with HFE mutations also meant removal of that person's respective reference individuals.

The combined cohort was linked to national, population-based registers. We used outcome data from the National Patient Register, the Causes of Death Register, the Prescribed Drug Register and the Cancer Register. These registers are described in more detail in the supplementary material Appendix, page 1. Briefly, the Causes of Death Register holds data on all causes of death in Sweden. The Cancer Register holds data on incident malignancies. These two registers were founded before the start of the study period, why data from these registers was available for the entire study period for all study persons. The National Patient Register was founded in the 1960s, with data on diagnoses from hospitalization events available for the full study period, with a separate Outpatient Register added in 1 January 2001, holding data on all contacts with specialized outpatient visits, but not including primary care. The Prescribed Drug Register was started in 30 July 2005 and holds prospective data on all dispensed drugs in Sweden, but not on pharmacological treatment in hospitals.

2.3 Outcomes

We considered the following outcomes: a diagnosis of HH, cirrhosis, HCC, breast cancer, colorectal cancer, type 1 and 2 diabetes, Parkinson's disease, osteoarthritis and hypothyroidism. Administrative codes used to define outcomes are listed in Table S1. Cancer outcomes were defined as the first occurrence of breast cancer, colorectal cancer or HCC in either the Patient Register, the Causes of Death register or the Cancer Register. We allowed persons to have multiple outcomes, e.g. a person could first have a breast cancer and later a colorectal cancer. These outcomes were available for the full study duration and included the full cohort.

Parkinson's disease and osteoarthritis were defined as a corresponding ICD-code (Table S1) in the Inpatient or Outpatient Register, or the Causes of Death Register. Cirrhosis was defined as a composite outcome, including the first diagnosis of either cirrhosis or a complication of cirrhosis, including ascites, oesophageal varices, hepatic encephalopathy or hepatorenal syndrome (definitions in Table S1). For these outcomes, we began follow-up at 1 January 2001 and excluded from all analyses study persons whose HFE was performed before that date in order to be able to use the Outpatient Register.

Type 1 and 2 diabetes and hypothyroidism are diseases that do not necessarily require inpatient care, or a specialist visit and are thus not captured by the Inpatient or Outpatients Registers, but most of these patients are treated with specific pharmacotherapies. Therefore, we defined these diseases as the first time a prescribed drug corresponding to the relevant disease was collected from a pharmacy by the patient. We used ATC codes in the prescribed drug register to define these outcomes as follows: For type 1 diabetes, we required a prescription for insulin (A10A, including subheadings) and age <40 years at the time of first prescription, and no code for other antidiabetic medications (A10B, including subheadings).

For T2D, we required no ICD diagnosis of type 1 diabetes at or prior to baseline, and an ATC code for other antidiabetics (A10B), or only a code for insulin (A10A) and age ≥40 years at the time of the first prescription. Hypothyroidism was defined as an ATC code for levothyroxine (H03AA01). For these outcomes, we excluded study persons with a baseline prior to 30 July 2005 (start of the Prescribed Drug Register). Persons with a dispensed drug up to 1 year after July 30, 2005 were counted as having the disease in question at baseline.

We evaluated prevalent and incident outcome diagnoses separately. Prevalent diagnoses were defined as having the diagnosis in question at or before the time of the HFE test, while incident outcomes were defined as receiving the diagnosis any time after the HFE test. This was done as the diagnosis in question might impact the reason for HFE testing, e.g. a person with cirrhosis is likely to be tested for HFE as part of the routine evaluation if other tests are negative. Persons with an outcome diagnosis present at or before baseline were excluded from analyses studying incident events for that particular outcome. We used the main cause of contact with the healthcare system to define outcomes, this was done to reduce the risk for differential misclassification.

2.4 Sensitivity analyses

Analyses were performed in several subgroups. First, we tested the hypothesis that the risk for incident malignancies compared to the matched reference population would be higher in persons with a homozygous p.C282Y mutation than in persons with a compound heterozygote p.C282Y/p.H63D mutation.

Second, we investigated risks stratified on gender as men are known to have a higher risk of developing phenotypic HH.

Third, in the subpopulations with available data on ALT or ferritin, we investigated if these biomarkers were associated with increased risk of the studied outcomes. ALT and ferritin were analysed as continuous parameters. These analyses were not performed on reference individuals since data on ALT or ferritin were not available.

Fourth, to further reduce the risk that prevalent diagnoses at baseline were included in the analysis investigating incident outcomes, we started follow-up in 2006, thus having a period of up to
5 years to better exclude any prevalent diagnoses at baseline using the outpatient register.

Fifth, as prevalent diagnoses might be the reason for why HFE testing was performed, for instance in the workup of a patient with newly diagnosed cirrhosis, we performed an analysis excluding those with the outcome-defining diagnosis made within 90 days prior to baseline, only counting diagnoses made before that timeframe.

Finally, we investigated if the risk for incident outcomes differed depending on if HH was diagnosed or not. For this analysis, we assumed that a person with a HFE mutation that had led to phenotypical HH at the time of testing would receive a HH diagnosis at least 1 year after baseline. We thus stratified the cohort by presence of a HH diagnosis up to 1 year after baseline and examined the risk of incident outcomes compared to reference individuals after that period. This analysis excluded any persons that died or were diagnosed with the outcome under study during that year.

2.5 Statistical analysis

Descriptive statistics are presented as medians and interquartile ranges for continuous data and as total numbers and percentages for categorical data. We used logistic regression to obtain odds ratios (OR) for prevalent disease at or before baseline, comparing persons with HFE mutations to reference individuals.

We used Cox proportional hazards regression to obtain hazard ratios (HR) as measures of relative risk in studying the association between the incident outcomes and the exposure variables. Individuals were followed until the event of interest, emigration from Sweden, death, or the end of follow-up, whichever occurred first. A person could contribute with multiple outcomes, for instance first hemochromatosis and later a diagnosis of cirrhosis. Attained age was used as the underlying time scale in all the models. The proportional hazards assumption was assessed both graphically and numerically using the Grambsch and Therneau test based on Schoenfeld residuals with no evidence of violation in any model except for the hypothyroidism outcome. To deal with the violation of proportional hazards assumption (P < .01) identified on one of the adjustment covariates (sex), we performed a sex stratified Cox regression analyses; this model gave a similar HR for the outcome variable of interest as in main model without stratification. All regression models were adjusted for age, sex and county of residence at baseline. STATA version 15.1 (StataCorp) and SAS (version 9.4) were used to perform all analyses.

2.6 Ethical considerations

This study was approved by the regional ethics committee in Stockholm (reg no 2015/1731-31/4). Informed consent was waived by the committee due to the retrospective data collection process.

### TABLE 1 Characteristics of the full cohort at baseline stratified on mutation type and sex

| Parameter | Complete data (n persons) | Median/number | IQR/% |
|-----------|--------------------------|---------------|-------|
| Age (y)   | 1610 596                  | 52.6          | 38.6-64.2 |
| Sex (female) | 1014                    |               | 44.6  |
| ALT (IU/L) | 700                      | 31            | 20-52 |
| Ferritin (µg/L) | 738                    | 610           | 293-1159 |
| Compound heterozygous p.C282Y/p.H63D (n = 1372) | 2035 2035 | 50.7  | 36.8-62.3 |
| Age (y)   | 1372 596                  | 51.3          | 36.5-62.3 |
| Sex (female) | 1372                    | 596           | 43.4  |
| ALT (IU/L) | 393                      | 32            | 21-53 |
| Ferritin (µg/L) | 438                    | 400           | 180-703 |
| Females (n = 1610) | 1610                    | 54.1          | 39.8-65.3 |
| ALT (IU/L) | 476                      | 24            | 16-36 |
| Ferritin (µg/L) | 514                    | 327.5         | 150-642 |
| Homozygous p.C282Y | 1610                    | 1014          | 63    |
| Females (n = 1610) | 1610                    | 596           | 37    |

### RESULT S

Baseline characteristics of the cohort at the time of HFE testing are presented in Table 1. We identified 3645 persons carrying homozygous or compound heterozygous HFE mutations. Median age was 52 years and 44% were females. The cohort was matched by age, sex and county of residence to 36 423 population-based reference individuals. In the HFE population, 2273 persons (62%) had a homozygous p.C282Y mutation, and 1372 persons (38%) had a compound heterozygous p.C282Y/p.H63D mutation. ALT could be retrieved in 30% of the cohort and ferritin in 32%. 3406 (93%) of all individuals were found having HFE mutations after 1 January 2001, and could thus contribute to assessment of outcomes using the Outpatient register. For outcomes using the Prescribed Drug Register, 2883 (79%) persons with HFE mutations were available for analysis, i.e., found having HFE mutations after July 30, 2005.
3.1 | Prevalent diagnoses at HFE testing

Diagnoses present at or before baseline in persons with HFE mutations and reference individuals with corresponding ORs are presented in Table 2. At the time of testing, a diagnosis of hereditary hemochromatosis (HH) was present in 287 cases (8.4%) of those with HFE mutations and in 12 (0.04%) reference individuals (aOR 265, 95%CI = 148-472, P < .001). Persons with a diagnosis of HH at the time of HFE testing had higher ferritin levels (median 647 µg/L, IQR = 312-1091) as compared to those without a HH diagnosis at baseline (median 435 µg/L, IQR = 164-868) (P < 0.001). Median ALT levels were similar across these groups (31 vs 32 IU/L, P = .4).

Cirrhosis (0.85% vs 0.13%, P < .01), HCC (0.05% vs 0.003%, P = .014), osteoarthritis (14.1% vs 9.5%, P < .01) and T2D (4.4% vs 3.7%, P = .036) were more common in persons with HFE mutations compared with reference individuals at baseline.

3.2 | Incident outcomes following a positive HFE test

Incident outcomes for the full cohort together with incidence rates per 1000 person-years of follow-up and adjusted HRs are presented in Table 3. The cohort was followed for a mean period of 7.9 years, equivalent to 313 197 person-years, with a maximal follow-up of 20 years. During follow-up, 339 persons (9.3%, incidence rate 12.3/1000 person-years) died, corresponding to a HR of 1.16 (95%CI = 1.04-1.30). There were 23 cases of HCC in persons with HFE mutations (0.63%) and 12 cases in reference individuals (0.03%), corresponding to an adjusted HR of 21.3 (95%CI = 10.3-44.0). The risk of cirrhosis was increased (aHR 2.15, 95%CI = 1.05-4.41), but was a rare outcome only found in 13 persons (0.38%) with HFE mutations and 60 reference individuals (0.18%).

Hereditary hemochromatosis was diagnosed during the full follow-up period in 1 694 (49.7%) persons with HFE mutations compared with 18 (0.05%) reference individuals (aHR 2318, 95%CI = 1281-4195).

A first-time diagnosis of osteoarthritis was less common after baseline in persons with HFE mutations compared with reference individuals (aHR 0.77, 95%CI 0.64-0.93). The risk for incident T2D was modestly increased (aHR 1.36, 95%CI 1.12-1.64).

We found no statistically significant difference compared to reference individuals in the risk for colorectal cancer (aHR 0.99, 95%CI = 0.67-1.46), breast cancer (aHR 1.08, 95%CI = 0.73-1.60), type 1 diabetes (aHR 2.34, 95%CI = 0.67-8.22), hypothyroidism (aHR 1.25, 95%CI = 0.99-1.58) or Parkinson’s disease (aHR 0.35, 95%CI = 0.11-1.10).

3.3 | Sensitivity analyses

3.3.1 | Incident outcomes in homozygous vs. compound heterozygous mutations

Incident outcomes together with incidence rates per 1000 person-years of follow-up and adjusted HRs are presented stratified by mutation type in Table S2a and S2b. An increased risk of HCC was found both in patients with homozygous p.C282Y (aHR 22.9, 95%CI = 10.4-50.6) and in compound heterozygous p.C282Y/p.H63D (aHR 15.5, 95%CI = 2.6-93.0), although most HCC cases (20/23, 87%) were found in the homozygous p.C282Y subgroup. The risk of T2D was increased in persons with a compound p.C282Y/p.H63D mutation (aHR 1.68, 95%CI 1.29-2.19) while no significantly increased risk was seen in persons with a homozygous p.C282Y mutation (aHR 1.12, 95%CI 0.85-1.47).

### TABLE 2 Prevalent diseases at or before baseline in persons with HFE mutations and in age, sex and living location-matched reference individuals with corresponding odds ratios

| Type of prevalent outcome (at baseline) | Persons with HFE mutations, n (included) | Reference individuals, n (included) | Persons with HFE mutations, n outcomes (%) | Reference individuals, n outcomes (%) | OR | 95% CI |
|----------------------------------------|------------------------------------------|-------------------------------------|------------------------------------------|-------------------------------------|-----|--------|
| Full cohort                            |                                           |                                     |                                          |                                     |     |        |
| Cirrhosis                              | 3406                                     | 34 023                              | 29 (0.85)                                | 43 (0.13)                           | 6.81| 4.24, 10.93 |
| HCC                                    | 3645                                     | 36 412                              | 2 (0.05)                                 | 1 (0.003)                           | 19.99| 1.81,220.51 |
| Colorectal cancer                      | 3645                                     | 36 412                              | 22 (0.60)                                | 275 (0.76)                          | 0.79| 0.51, 1.23 |
| Breast cancer                          | 3645                                     | 36 412                              | 48 (1.32)                                | 480 (1.32)                          | 1.00| 0.74, 1.35 |
| Type 1 diabetes                        | 2883                                     | 28 801                              | 42 (1.46)                                | 430 (1.49)                          | 0.97| 0.71, 1.34 |
| Type 2 diabetes                        | 2883                                     | 28 801                              | 128 (4.44)                               | 1057 (3.67)                         | 1.23| 1.01, 1.48 |
| Hypothyroidism                         | 2883                                     | 28 801                              | 54 (1.87)                                | 455 (1.58)                          | 1.19| 0.89, 1.59 |
| Parkinson’s disease                    | 3406                                     | 34 023                              | 11 (0.32)                                | 81 (0.24)                           | 1.36| 0.72, 2.56 |
| Osteoarthritis                         | 3406                                     | 34 023                              | 480 (14.09)                              | 3231 (9.50)                         | 1.60| 1.44, 1.77 |
| Hemochromatosis                        | 3406                                     | 34 034                              | 287 (8.43)                               | 12 (0.04)                           | 264.63| 148.33, 472.13 |

Abbreviations: CI, confidence interval; DM, diabetes mellitus; HCC, hepatocellular carcinoma; OR, odds ratio.
3.3.2 | Incident outcomes in men vs women

Data on risk for incident outcomes in men and in women are presented in Table S3. At baseline, women were older (median 54.1 years, IQR = 39.7-65.2) compared to men (50.7, IQR = 36.8-62.3, P < .01). The fraction of individuals with the p.C282Y homozygous mutation was similar in women (63%) and men (62%) (P = 0.68). Overall mortality compared to reference individuals was increased in men (aHR 1.30, 95%CI = 1.12-1.50) but not in women (aHR 0.98, 95%CI = 0.82-1.18). The risk of HCC was relatively higher in men (aHR = 2.26, 95%CI = 1.02-5.00) than in women (aHR = 1.54, 95%CI = 0.97-2.41).

3.3.3 | Impact of ALT and ferritin on incident outcomes

A higher ALT was associated with an increased risk for T2D (aHR = 4.53, 95%CI = 2.40-8.56 per unit [μkat/L] increase) and overall mortality (aHR 2.10, 95%CI = 1.23-3.58, P = .006). No other associations were statistically significant.

For ferritin, associations were found with incident HCC (aHR = 1.001 per unit [μg/L] increase, 95%CI = 1.0003-1.001, P = .003) and hemochromatosis. These data are presented in Table S4.

3.3.4 | Starting follow-up in 2006

The analysis with an extended wash-out period found similar risks as in the main analysis (Table S5).

3.3.5 | Excluding prevalent diagnoses up to 90 days prior to baseline

The risk of incident outcomes was examined in persons with a HH diagnosis up to 1 year after baseline compared to reference individuals, and in persons not receiving a HH diagnosis up to 1 year after baseline. Of all persons with HFE mutations and a subsequent diagnosis of HH, 1652/1694 (97.5%) were diagnosed with HH up to 1 year after baseline. The risk of incident outcomes was generally reduced compared to the main analysis for the following diagnoses: cirrhosis (aOR = 2.00 compared to aOR = 6.81; HCC (aOR = 1.07 vs aOR = 1.00) compared to aOR = 19.99 compared to aOR = 6.81); HCC (aOR = 10.7 vs aOR = 10.0) compared to aOR = 19.99; and osteoarthritis (aOR = 1.07 vs aOR = 1.00) compared to aOR = 19.99.

3.3.6 | Impact of a hereditary HH diagnosis

The risk of incident outcomes was increased in patients with a HH diagnosis up to 1 year after baseline compared to reference individuals matched for age, sex and living location. The ORs for these sensitivity analyses were generally reduced compared to the main analysis for the following diagnoses: cirrhosis, osteoarthritis, hypothyroidism, cirrhosis, and osteoarthritis. These data are presented in Table S6.

### Table 3: Incident outcomes, hazard ratios and incidence rate of outcomes in patients with a HFE mutation (n = 3645) and reference individuals matched for age, sex and living location

| Incident outcomes (after HFE mutation diagnosis) | Persons with HFE mutations, N (included) | Reference individuals, N (included) | Events in persons with HFE mutations, n (%) | Events in reference individuals, n (%) | Incidence rate per 1000 person-years, persons with HFE mutations (95% CI) | Incidence rate per 1000 person-years, reference individuals (95% CI) | HR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| HCC | 3645 | 36412 | 23 (0.63) | 12 (0.03) | 0.80 (0.53, 1.21) | 0.04 (0.02, 0.07) | 21.32 | 10.34, 43.97 |
| Colorectal cancer | 3645 | 36412 | 50 (1.37) | 570 (1.57) | 1.03 (0.71, 1.49) | 1.05 (0.94, 1.18) | 0.99 | 0.67, 1.46 |
| Breast cancer | 3645 | 36412 | 73 (2.00) | 742 (2.04) | 1.03 (0.71, 1.50) | 0.95 (0.84, 1.07) | 1.08 | 0.73, 1.60 |
| Type 1 DM | 2883 | 28801 | 6 (0.21) | 79 (0.27) | 0.18 (0.06, 0.56) | 0.08 (0.04, 0.13) | 2.34 | 0.67, 8.22 |
| Type 2 DM | 2883 | 28801 | 283 (9.82) | 2392 (7.96) | 7.57 (5.37, 9.05) | 5.72 (3.37, 9.09) | 1.36 | 1.12, 1.64 |
| Hypothyroidism | 2883 | 28801 | 278 (9.64) | 2101 (7.29) | 4.90 (3.92, 6.12) | 3.92 (3.63, 4.23) | 1.25 | 0.99, 1.58 |
| Parkinson’s disease | 3406 | 34023 | 7 (0.21) | 142 (0.42) | 0.13 (0.04, 0.39) | 0.37 (0.30, 0.45) | 0.35 | 0.11, 1.10 |
| Cirrhosis | 3406 | 34023 | 13 (0.38) | 60 (0.18) | 0.38 (0.20, 0.73) | 0.18 (0.13, 0.24) | 2.15 | 1.05, 4.41 |
| Osteoarthritis | 3406 | 34023 | 293 (8.60) | 3292 (9.68) | 5.15 (4.29, 6.19) | 6.71 (6.38, 7.05) | 0.77 | 0.64, 0.93 |
| Hemochromatosis | 3406 | 34034 | 1694 (49.7) | 18 (0.05) | 119.01 (113.11, 125.22) | 0.04 (0.02, 0.08) | 2317.90 | 1280.75, 4194.92 |
| Death | 3645 | 36412 | 339 (9.30) | 3149 (8.65) | 12.29 (11.05,13.67) | 11.01 (10.64,11.41) | 1.16 | 1.04, 1.30 |

Abbreviations: CI, confidence interval; DM, diabetes mellitus; HCC, hepatocellular carcinoma; HR, hazard ratio.
1 year after the date of HFE testing. Estimates from this analysis are presented in Table S7.

4 | DISCUSSION

In this large cohort study, we could confirm the findings from a recent population-based study, linking the homozygous p.C2828Y or compound heterozygous p.C2828Y/p.H63D genotypes to increased risk of cirrhosis, HCC, T2D and osteoarthritis, but did not detect any increased risk for colorectal or breast cancer. In absolute terms, however, liver-related outcomes were rare, affecting only around 1% of the cohort. Men with HFE mutations had an increased mortality compared to reference individuals, while no increased risk was found in women. Our finding of no significant increased risk for colorectal or breast cancer in HFE mutations carriers is in contrast to previous studies. Likewise, the risk of developing Parkinson’s disease was not altered in our main analysis, in contrast to other findings describing a decreased risk. This discrepancy could be due to the size of this study together with systematic ascertainment of outcomes from national registers.

Our risk estimates were modified by mutation type and gender for some outcomes, which might be due to lower power of each analysis but could also have biological causes. For instance, the elevated risk of overall mortality was not found in women, which is biologically plausible since women develop symptomatic HH at a later age compared to men. These results can be used to inform care of patients with HFE mutations regarding the future risk of the studied outcomes.

In an analysis of participants in the UK biobank, of 2890 persons found to be homozygous for p.C282Y, 22% of men and 10% of women developed HH after 7 years of follow-up. This is lower compared to our findings, where 53% of men and 46% of women not being diagnosed with HH at baseline were diagnosed with incident HH after a similar follow-up of 7 years. This high penetration of HH could possibly be due to examining a slightly different population, since patients in our study were likely tested as part of a liver work-up or a suspicion of HH, while participants in the UK biobank, by contrast, could be more healthy than the general population since they volunteered to participate. The risk of overall mortality was slightly increased in our study as compared to non-significant after adjustments in the UK biobank study, and we found that the risk for excess mortality at around 7 years of follow-up was restricted to men. With respect to HH, diabetes, osteoarthritis, cirrhosis and HCC, our findings of an increased risk are comparable to those of the UK biobank study, that did not examine the risk of colorectal and breast cancer.

Our study is one of the largest cohorts on the long-term outcomes of persons with homozygous or compound heterozygous HFE mutations. Our large sample size provided estimates that were more precise than most previous results. The study participants were derived from laboratories reporting to all university hospitals in Sweden, increasing internal validity. Although we could not capture HFE cases diagnosed at laboratories outside of university hospitals, the low number of HH diagnoses in the reference population (0.05% of 36 423 persons) suggests that we likely captured most cases. The central registers of Sweden allowed us to identify, in effect, all outcomes during the study period, with very little loss to follow-up, and independently of the exposure (a positive HFE test).

A main limitation of this study is that we do not know the precise reason for HFE testing. This could be part of a work-up for suspected liver disease or HH, or family screening of HH patients. Indeed, the data suggests that for cirrhosis, HCC and osteoarthritis, symptomatic disease was likely to be the reason for the HFE testing as in many cases the HFE testing was performed within 90 days of the diagnosis in question. The high penetrance of a HH diagnosis in this cohort could possibly also be attributed to the level of care given to these patients, although we cannot know if the HH diagnosis is correct in all cases. Further, we lack detailed data on the magnitude of iron overload, in which cases where treatment with phlebotomy was initiated, and if this affected the outcomes.

Taken together, our results should be generalizable to HFE carriers diagnosed at a secondary or tertiary level of care and thereby relevant for clinicians working in such hospitals. Multiple comparisons across subgroups increases the risk of type 1 and type 2 errors, which is why our results from the subgroup analyses should be interpreted carefully, particularly for rare outcomes. Other limitations include the lack of serum ALT and ferritin data in around 70% of the cohort, as well as in the reference population. Finally, it is possible that there is residual confounding in parameters such as alcohol consumption between subgroups (perhaps in particular between men and women) that could affect the estimates.

5 | CONCLUSIONS

In a large cohort of individuals with homozygous or compound heterozygous HFE mutations diagnosed at secondary or tertiary level of care, approximately 50% were diagnosed with hereditary HH after in mean 7.9 years of follow-up. An increased risk of cirrhosis and liver cancer was found, but these outcomes were rare, affecting only 1% of the cohort. The risk of overall mortality was increased also in HFE carriers without a diagnosis of hereditary HH. We found increased risks for development of diabetes type 2 and osteoarthritis, but the risk of colorectal and breast cancer development was not increased. The findings can be used to inform clinicians about the natural history of patients with HFE mutations and generate hypotheses for future research.

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CONFLICT OF INTEREST

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**AUTHOR CONTRIBUTIONS**

HH, PS: Study conception and design; HH, MJ, ME, IP, FR, NN, DK, MW, HUM, PS: Acquisition of data; NN: Statistical analysis. All are involved in analysis, interpretation of data and critical revision. HH, PS: Drafting of manuscript. HH: Guarantor of article. All authors approved the final version of the article, including the authorship list.

**ORCID**

Hannes Hagström  [ORCID](https://orcid.org/0000-0002-8474-1759)

Nelson Ndewa  [ORCID](https://orcid.org/0000-0001-5297-5107)

Hanns-Ulrich Marschall  [ORCID](https://orcid.org/0000-0001-7347-3085)

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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