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Risk of cancer by transferrin saturation levels and haemochromatosis genotype: population-based study and meta-analysis

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Abstract. Ellervik C, Tybjærg-Hansen A, Nordestgaard BG (Herlev Hospital, Herlev; Naestved Hospital, Naestved; Copenhagen University Hospitals and Faculty of Health Sciences, Copenhagen; Rigshospitalet, Copenhagen; The Copenhagen City Heart Study, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark). Risk of cancer by transferrin saturation levels and haemochromatosis genotype: population-based study and meta-analysis. *J Intern Med* 2012; **271**: 51–63.

Objective. Increased iron overload, whether or not owing to the presence of the haemochromatosis genotype C282Y/C282Y, may be associated with an increased risk of cancer. The aim of this study was to test the hypothesis that elevated transferrin saturation levels (as a proxy for iron overload) and haemochromatosis genotype C282Y/C282Y are associated with an increased risk of cancer.

Methods. We conducted a population-based study of 8763 individuals, of whom 1417 developed a first cancer during 15 years of follow-up, and a meta-analysis. We stratified absolute 10-year risk of cancer by smoking status, an important risk factor.

Results. In women, transferrin saturation above 60% versus below 50% was associated with a hazard ratio of 3.6 (95% confidence interval (CI): 2.0–6.5; $P < 0.001$) for any cancer; risk of liver cancer was increased in both women and men. In women, the corresponding absolute 10-year risk of any cancer was 34% and 30% in smokers and nonsmokers, respectively. In men, haemochromatosis genotype C282Y/C282Y versus wild type/wild type was associated with a hazard ratio of 3.7 (95% CI: 1.2–12; $P = 0.01$) for any cancer, with a similar trend in women. In men, the corresponding absolute 10-year risk of cancer was 39% and 27% in smokers and nonsmokers, respectively. Other haemochromatosis genotypes were not associated with increased risk of cancer in women or men. From the meta-analysis, the odds ratio of any cancer for transferrin saturation ≥60% versus a reference group was 1.5 (95% CI: 1.2–1.8) for women and men combined.

Conclusions. We have demonstrated that elevated transferrin saturation levels in women and haemochromatosis genotype C282Y/C282Y in men are associated with increased risk of cancer. Thus, our results support the implementation of cancer screening programmes in patients with iron overload or with C282Y/C282Y.

Keywords: cancer, follow-up, haemochromatosis genotype, meta-analysis, transferrin saturation.

Introduction

Iron-induced free radical damage to DNA may be important for the development of cancer [1], and cancer cells may grow more rapidly in response to increased iron levels [2]. Thus, iron overload, whether or not owing to the presence of the haemochromatosis genotype C282Y/C282Y, may lead to an increased risk of cancer. Homozygosity for C282Y in the *HFE* gene explains 83% of hereditary haemochromatosis [3], an autosomal recessive trait causing iron overload and lifelong iron accumulation in various organs [4]. The most severe outcome of hereditary haemochromatosis is liver cancer [4], which has been associated with haemochromatosis genotype C282Y/C282Y in case–control studies [5].

The risk of cancer in individuals with iron overload has previously been studied in various prospective [6–17] and case–control studies [18], but results have been inconsistent. No population-based follow-up studies of cancer risk according to haemochromatosis genotype have been conducted.

The aim of this study was to test the hypothesis that elevated transferrin saturation levels (as a proxy for iron overload) and haemochromatosis genotype C282Y/C282Y are associated with an increased risk of cancer. For this purpose, we used a population-based study of 8763 individuals, of whom 1417 developed a first cancer during 15 years of follow-up. We stratified absolute 10-year risk by smoking status, an important cancer risk factor [19]. Furthermore, we conducted a meta-analysis of risk of cancer according to elevated transferrin saturation from prospective studies including 57 841 individuals.

Materials and methods

Participants

Using data from a prospective study of the Danish general population, the Copenhagen City Heart Study (CCHS), we examined 8763 subjects aged 21–93 years and with no history of cancer who participated in the initial 1991–1994 examination [20]. Participants were individuals randomly selected on the basis of the Danish Central Population Register Code to reflect the adult general population. The study was approved by Herlev Hospital and a Danish ethical committee (No. KF-100.2039/91). Written informed consent was obtained from participants. The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. All participants were white and of Danish descent.

Diagnoses of incident cancer for the whole cohort from 1947 until 2007 were obtained from the national Danish Cancer Registry [21, 22], which identifies 98% of all cancers in Denmark [23], and the national Danish Patient Registry. Cancer diagnoses were classified according to criteria from the World Health Organization International Classification of Diseases 7th edition (ICD-7) and divided into 27 different subtypes [24]. Follow-up ended at the time of death, emigration or event or on 11 August 2007, whichever was first. During the period from study entry in 1991–1994 until the end of follow-up, 2233 cancers developed as follows: oral cavity/pharynx (n = 43), oesophagus (n = 34), stomach (n = 41), colon/rectum/anus (n = 234), liver/biliary tract (n = 47), pancreas (n = 63), larynx (n = 24), lung (n = 325), skin (n = 72), breast (n = 236), cervix uteri (n = 16), corpus uteri (n = 46), ovary (n = 62), prostate (n = 127), testis (n = 0), bladder/urinary tract (n = 123), kidney (n = 36), brain/nervous tissue (n = 46), thyroid/other endocrine tumours (n = 6), non-Hodgkin lymphoma (n = 51), Hodgkin's disease (n = 2), multiple myeloma (n = 21), leukaemia (n = 53), nonmelanoma skin (n = 338), sarcoma/other mesodermal tumours (n = 19), metastatic disease (n = 122) and others (including small intestine) (n = 46). Among study participants who developed more than one cancer, 1417 had a first cancer during follow-up. For cancer subgroup analyses, gastrointestinal cancer included the following: cancers of the oral cavity/pharynx, oesophagus, stomach, colon/rectum/anus, liver/biliary tract or pancreas; female cancer included the following: cancers of the breast, cervix uteri, corpus uteri or ovary; male cancer included cancers of the prostate or testis; respiratory cancer included cancers of the larynx or lung; haematological cancer included the following: non-Hodgkin's lymphoma, Hodgkin's disease, multiple myeloma or leukaemia; urinary cancer included cancers of the bladder/excretory urinary tract or kidney; other cancer included the remaining cancer subgroups. Follow-up was 100% complete.

Blood samples

Blood samples were collected under nonfasting conditions between 8 am and 4 pm. Transferrin saturation (%) was determined as iron level (in μmol L^{-1}) divided by 2 × transferrin level (in μmol L^{-1}) × 100. The transferrin level was measured by nephelometry [Behring Nephelometer Analyzer II. (Dade Behring, Deerfield, IL, USA)] and iron level by colorimetry (Konelab autoanalyzer; ThermoFisher Scientific Inc., Waltham, MA, USA). Transferrin saturation was only measured once, and data were available for 8039 individuals. In addition, standard hospital assays were used to measure plasma levels of alanine amino transferase (U L^{-1}), gamma glutamyl transferase (U L^{-1}), bilirubin (μmol L^{-1}) and albumin (μmol L^{-1}) (Konelab, Helsinki, Finland or Boehringer Mannheim, Mannheim, Germany).

Genotyping

Genotyping for C282Y [the Single Nucleotide Polymorphism Database (dbSNP): rs1800562] and H63D (dbSNP: rs1799945) was by allele-specific amplification, with restriction enzyme digestion to confirm genotype [25]. The genotypes were in Hardy–Wein-

berg equilibrium, and data were available for 8285 individuals. Clinically overt haemochromatosis was not diagnosed in any of the 21 C282Y/C282Y individuals before or after study entry. Three of the 21 C282Y/C282Y individuals had been blood donors. In 2001, all living C282Y/C282Y individuals were informed of their genotype status as well as the possibility of a slightly increased risk of developing haemochromatosis and the option of treatment with phlebotomy. Of the 20 C282Y/C282Y individuals still alive in 2001, all were then referred to a hospital for the management of and possible treatment for haemochromatosis. Whether or not C282Y/C282Y individuals were treated with phlebotomy from 2001 onwards was not recorded.

Other characteristics

Individuals were questioned about alcohol consumption, smoking habits, the number of previous pregnancies and the use of oral contraceptive drugs and hormone-replacement therapy. Body mass index was calculated as weight in kilograms divided by height in metres squared. Further details are described in the statistical analyses section below.

Statistical analyses

stata/se 10.0 statistical software package was used for analyses. Two-sided $P < 0.05$ was considered significant. Bonferroni correction was used for multiple comparisons of genotype results, with five genotype comparisons for each endpoint ($P \leq 0.01$ was considered significant). Mann–Whitney *U* test was used for continuous variables, and Pearson's chi-square test was used for categorical variables. All analyses were stratified a priori by gender, because the clinical penetrance of haemochromatosis differs markedly between men and women.

Cumulative incidences were plotted with the use of Kaplan–Meier curves, and differences between transferrin saturation levels or genotypes were examined by log-rank tests. Cox proportional hazards regression was used to estimate hazard ratios with 95% confidence intervals (CIs). Age at event was analysed using left truncation (or delayed entry) and age as the timescale. Thus, age is automatically adjusted for, and it is assumed that there may have been a period before study entry in which the individual was subjected to the effects of elevated transferrin saturation levels or genotype. With age as the timescale, we cannot study the effects of age itself. Therefore, for the test of interaction of age with transferrin saturation levels or genotype, we used years of follow-up as the timescale analysing time to event. No significant or clinically relevant interactions were observed. The assumption of proportional hazards was tested using Schoenfeld residuals, and no violations were observed. Transferrin saturation levels were coded as follows: (i) (<50%), (ii) (50% to <60%) and (iii) (≥60%) for the trend tests in log-rank and Cox analyses. Interaction of transferrin saturation levels or genotypes with other risk factors was evaluated by including two-factor interaction terms, one at a time, in the multifactorial Cox regression model. Cox regression analyses included adjustment for competing risks of death by censoring individuals at the date of death irrespective of the cause and by adjusting for common risk factors for competing risks of death (see Table 1), as discussed later.

Multifactorially adjusted models also included time-dependent covariates from the 1991–1994 examination and the 2001–2003 re-examination. If only baseline values (1991–1994) were available, these were used for adjustment during the entire follow-up period. Multifactorial adjustments included for age, alcohol consumption (intake of ≤1 drink/day vs. >1 drink/day), smoking habits (current vs. nonsmoker; pack-years of smoking: 0, ≤10 (but >0) and >10, where 1 pack-year = 1 pack of 20 cigarettes/day for 365 days year^{-1}), body mass index (<25 vs. ≥25 kg m^{-2}) and, for women, nulliparity (yes/no), use of oral contraceptive drugs (yes/no) or hormonal-replacement therapy (yes/no) and menopausal status (premenopausal/postmenopausal). In analyses of transferrin saturation, alcohol intake was stratified for, a priori (and not included as an adjustment variable), as excessive alcohol intake may increase transferrin saturation levels [26].

Absolute risks for cancer by transferrin saturation levels or genotypes were estimated using the regression coefficients from a Poisson regression model with smoking (yes/no) as a covariate. Absolute risks are presented as estimated incidence rates (events/10 years) in percentages. Population attributable risk was estimated as [f (HR-1)]/[1-f(HR-1)], where f is the frequency of transferrin saturation ≥60% or C282Y/C282Y in the population, and HR is the hazard ratio for cancer [27].

Meta-analysis

Search strategy and selection criteria. Prospective human studies published until 10 September 2009

Table 1 *Baseline characteristics of participants from the general population with and without cancer developed during 15 years of follow-up*

	Women		Men	
	Cancer		Cancer	
Characteristic	No	Yes	No	Yes
Number, *n*	4114	747	3232	670
Age, years	59 (45–70)	67 (58–73)***	55 (43–66)	66 (59–72)***
Transferrin saturation, %	25 (20–32)	26 (21–32)	29 (23–36)	30 (23–37)
Wild type/wild type, %	66.5	67.9	67.3	64.3
H63D/wild type, %	20.6	21.2	20.2	21.8
H63D/H63D, %	1.7	2.6	1.6	1.9
C282Y/wild type, %	9.2	6.6	9.7	10.5
C282Y/H63D, %	1.7	1.3	1.2	1.1
C282Y/C282Y, %	0.3	0.4	0.1	0.5
Total tobaccco consumption, pack-years[a]	7 (2–15)	8 (2–19)	15 (6–30)	25 (11–41)
Current smoker, %	45	50*	51	61***
Body mass index, kg m^{-2}	24 (22–28)	25 (22–228)*	26 (23–28)	26 (24–28)**
Alcohol, g $week^{-1}$	36 (0–96)	36 (0–96)	120 (48–228)	132 (48–252)*
Oral contraceptive use, % of women	5	1***	–	–
Hormone replacement therapy, % of women	15	22***	–	–
Nulliparity, % of women	26	17***	–	–
Plasma alanine amino transferase, U L^{-1}	11 (8–15)	11 (9–16)	14 (10–21)	13 (9–20)*
Plasma gamma glutamyl transferase, U L^{-1}	26 (20–38)	27 (21–43)***	37 (27–58)	41 (30–66)***
Plasma bilirubin, μmol L^{-1}	9 (8–12)	9 (8–12)	10 (8–14)	10 (8–13)
Plasma albumin, μmol L^{-1}	630 (598–662)	617 (585–646)***	645 (611–678)	626 (595–657)***

Of the 8763 individuals, 8039 had a measurement of transferrin saturation and 8285 had a hemochromatosis genotype.
Variables expressed as median (± interquartile range) or proportion were collected at the 1991–1994 examination of the Copenhagen City Heart Study.
Statistical comparisons were made using two-sided Mann-Whitney *U* test, and Pearson's χ^2 test as appropriate: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
[a]Pack-years indicate the average number of packs of cigarettes smoked daily by a person multiplied by the number of years that person has been a smoker.

on the risk of any cancer by elevated transferrin saturation or C282Y/C282Y genotype were selected. A search was performed on PubMed and EMBASE and through scanning of reference lists of identified articles for relevant studies.

The keywords used were (hits from Pubmed displayed) as follows: (i) (cancer OR malignancy) AND transferrin saturation AND (follow-up OR prospective), 26 hits, 6 retrieved [7, 8, 10, 13, 14, 16]; (ii) (Cancer OR malignancy) AND C282Y AND (follow-up OR prospective), nine hits, none retrieved. A search on EMBASE did not provide any additional articles. Another reference [11] was retrieved from a search of journals by hand.

Studies were included if they were prospective with follow-up data (i.e. incidence) of total cancer as the endpoint and provided risk estimates with confidence limits or tabular data. Studies were excluded if they only provided cancer mortality incidence or were duplicates.

Data abstraction. The following information was abstracted from each study according to a fixed protocol: transferrin saturation, risk estimate and confidence limits, adjustment variables, tabular data if provided and age, sex, geographical location and ethnic group of the participants. There were no prospective studies on C282Y/C282Y genotype and risk of cancer.

Statistical analysis. Statistical analyses were performed with Stata (version 10.0) statistical software with the Meta command calculating both fixed and random effect measures from reports of effect measures and confidence intervals [28]. Statistical heterogeneity was assessed by the Q statistic with a corresponding *P*-value, although the power of this statistic is low with only a few studies [29]; $P < 0.05$ was considered significant. Owing to very few studies in the meta-analyses ($n = 3$), it was not possible to assess publication bias. Methodological heterogeneity was assessed a priori by stratification according to sex.

Results

Table 1 shows the characteristics of the participants at study entry. The median follow-up time was 14 years (interquartile range, 13–15 years).

Risk of any cancer

Transferrin saturation. In women, cumulative incidence by age of any cancer increased stepwise with increasing transferrin saturation level (Table 2, $P < 0.0001$). The highest risk was observed in women with transferrin saturation ≥60% vs. <50% with a multifactorially adjusted hazard ratio of 3.6 (95% CI: 2.0–6.5; $P < 0.001$). The corresponding results in men and for the combined analysis of both women and men were not significant; however, the study lacked statistical power to exclude the possibility of a 1.9-fold and a 1.8-fold or smaller increased risk of any cancer in men or in men and women combined, respectively, with transferrin saturation ≥60% vs. <50%. The incidence rate of cancer in the 0.7% ($n = 24$) of men ($n = 3530$) with the highest transferrin saturation level (≥75%) was 13 events/1000 person-years (95% CI: 5–34), which is similar to the incidence rate of 14 (95% CI: 13–15) in men with transferrin saturation <50%. Stratification of the results shown in Table 2 according to alcohol consumption gave similar results for high and low alcohol intake (data not shown). Also, the results shown in Table 2 were similar when participants aged <40 years were excluded from the analysis (data not shown).

In women, absolute 10-year risk of any cancer increased with increasing levels of transferrin saturation and smoking (Fig. 1, left panel). Women with transferrin saturation levels ≥60% had the highest absolute 10-year risk of cancer of 34% and 30% in smokers and nonsmokers, respectively. In men, absolute 10-year risk of any cancer did not increase with increasing levels of transferrin saturation, but it did increase with smoking.

Based on a frequency of 0.7% for transferrin saturation ≥60% in women in the general population and a multifactorially adjusted hazard ratio of 3.6 for any cancer, the population attributable risk was 2%. Risk of any cancer by transferrin saturation levels in quartiles did not reveal any significant difference in any quartile or any trend across strata, in women, men or in women and men combined (Supplementary Table S1).

Haemochromatosis genotype. In men, cumulative incidence by age of any cancer was higher in individuals with C282Y/C282Y versus wild type/wild type (Table 3, $P = 0.02$). The multifactorially adjusted hazard ratio of any cancer in men with C282Y/C282Y versus wild type/wild type was 3.7 (95% CI: 1.2–12; $P = 0.01$), with a similar trend in women. The hazard ratio value was also significant after correction for multiple comparisons. The study lacked the power to exclude the possibility that women with C282Y/C282Y have a 2.8-fold or less increased risk of any cancer. Other haemochromatosis genotypes were not associated with an increased risk of cancer in women or men (Table 3).

The multifactorially adjusted hazard ratio of any cancer for the combined analysis of women and men with C282Y/C282Y versus wild type/wild type was 2.4 (95% CI: 1.1–5.3; $P = 0.04$) (Table 3).

In men, absolute 10-year risk of any cancer was increased for the C282Y/C282Y genotype with the highest risk of 39% found among current smokers and 27% in nonsmokers (Fig. 1, right panel). Similar trends were seen in women.

In the six individuals with C282Y/C282Y who developed cancer during the follow-up period, median baseline levels of transferrin saturation and ferritin were 74% (interquartile range 55–79) and 603 $\mu g\ L^{-1}$ (322–646), respectively, (Supplementary Table S2). Median exposition time for cancer since study entry was 4 years. None of these six individuals developed liver cancer.

Based on a frequency of 0.2% for C282Y/C282Y in men in the general population and a multifactorially adjusted hazard ratio of 3.7 for any cancer, the population attributable risk was 0.5%.

Table 2 *Risk of any first cancer according to transferrin saturation levels*

	Transferrin saturation, groups	Transferrin saturation, IQR	Participants *N*	Events *N*	Incidence rate (95% CI) Events/1000 person-years	Log-rank *P*-value	Hazard ratio (95% CI) Age adjusted	*P*-value	Multifactorial adjustment	*P*-value	Power
Women	<50%:	20–31%	4406	672	12.0 (11.1–13.0)	–	1.0	–	1.0	–	–
	≥50% & <60%	51–56%	72	17	19.9 (12.4–32.1)	0.009	1.9 (1.2–3.0)	0.01	1.8 (1.1–2.9)	0.01	2.2
	≥60%	65–80%	31	11	32.7 (18.1–59.1)	<0.0001	3.4 (1.9–6.2)	<0.001	3.6 (2.0–6.5)	<0.001	2.8
P-trend	Total		4509	700	<0.0001		<0.001		<0.001		
Men	<50%	23–35%	3327	567	14 (13–15)	–	1.0	–	1.0	–	–
	≥50% & <60%	52–56%	129	22	14 (9–21)	0.9	1.0 (0.7–1.6)	0.94	1.0 (0.6–1.5)	0.76	1.7
	≥60%	65–79%	74	11	12 (6–21)	0.4	0.8 (0.4–1.4)	0.43	0.8 (0.4–1.4)	0.23	1.9
P-trend	Total		3530	600	0.70		0.73		0.24		
All	<50%	20–33%	7733	1239	13 (12–13)	–	1.0	–	1.0	–	–
	≥50% & <60%	51–56%	201	39	16 (12–22)	0.24	1.3 (0.9–1.8)	0.12	1.2 (0.9–1.7)	0.20	1.5
	≥60%	65–79%	105	22	17 (11–26)	0.14	1.3 (0.8–2.0)	0.23	1.3 (0.8–2.0)	0.23	1.8
P-trend	Total		8039	1300	0.16		0.07		0.09		

IQR, interquartile range.
All participants were free of cancer at study entry.
Multifactorial adjustment was for age, gender (only 'All'), body mass index, tobacco consumption, smoking habits and alcohol consumption.
For women nulliparity, use of hormone replacement therapy and use of oral contraceptive drugs were also included.
Power: 80% power to detect the hazard ratio shown at two-sided $P = 0.05$.

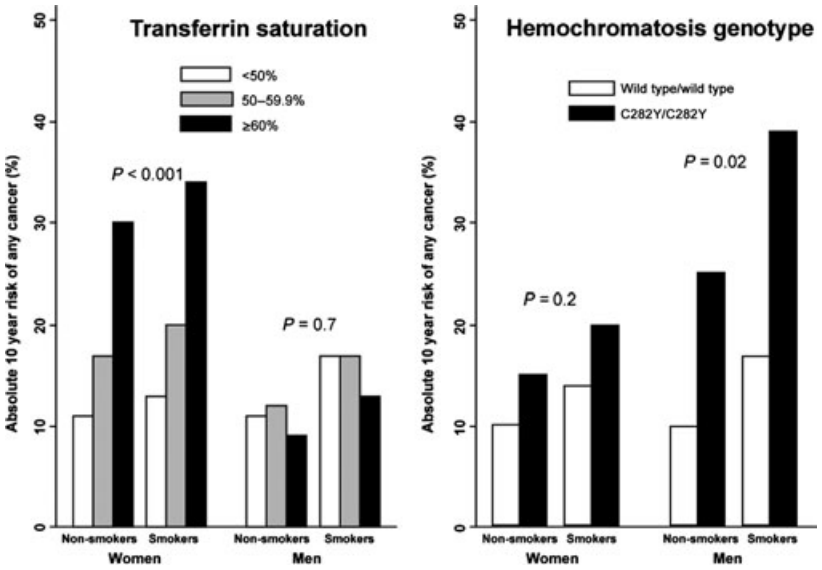

Fig. 1 *Absolute 10-year risk of any cancer by transferrin saturation levels and haemochromatosis genotype C282Y/C282Y. Based on 8763 individuals from the Copenhagen City Heart Study followed for 15 years, during which time 1417 developed cancer.*

Cancer subgroups

Results for exploratory analyses among five major cancer subgroups are shown in Table 4; however, the statistical power of these analyses is limited. In women, we observed a multifactorially adjusted hazard ratio for female cancer of 3.2 (95% CI: 1.3–7.7; $P = 0.01$) for transferrin saturation ≥60% vs. <50%. Also, risk of liver cancer was increased in both women and men with transferrin saturation ≥60% vs. <50%.

Meta-analysis

The odds ratio of any cancer for transferrin saturation ≥60% versus a reference group was 1.5 (95% CI: 1.2–1.8) for women and men combined with both fixed and random effect models (Fig. 2). Corresponding odds ratios for fixed and random effect models were 2.0 (95% CI: 1.5–2.7) and 2.2 (95% CI: 1.2–3.8) in women alone and 1.3 (95% CI: 1.1–1.6) and 1.3 (95% CI: 1.0–1.8) in men alone, respectively. Data for individual studies included and excluded are shown in Supplementary Table S3.

Discussion

To our knowledge, this is the first population-based follow-up study of transferrin saturation levels and haemochromatosis genotype including estimation of both relative and absolute risks of any cancer. We found that elevated transferrin saturation levels in women and haemochromatosis genotype C282Y/C282Y in men were associated with increased risk of cancer. In the meta-analysis, association between elevated transferrin saturation and increased risk of any cancer was found overall, in women and, to a lesser degree, in men.

Owing to the age range of participants, it is not surprising that within a small population with haemochromatosis genotype C282Y/C282Y ($n = 21$ in total including 14 women, in whom low penetrance is because of physiological causes) many participants did not have high levels of iron in the body; thus, our sex-stratified analyses of C282Y/C282Y participants may be underpowered. The prevalence of C282Y homozygosity among men (0.19%) was less than in women (0.31%) in our study. If men with C282Y/C282Y have a higher rate of cancer, as appears to be the case from our study, such men may be too ill to participate in this general population study or may even have died prematurely. This may explain the larger number of women with C282Y/C282Y compared with men in the study, as well as the fact that the percentage of men with cancer associated with elevated transferrin saturation was lower than that of women.

A biologically plausible mechanism for the association between elevated transferrin saturation and cancer risk may be iron-induced increased oxidative stress via the Fenton reaction [1], followed by promotion of the development of cancer cells. Likewise, oxidative stress has been shown to be increased in

Table 3 *Risk of any first cancer according to hemochromatosis genotype*

Genotype	Participants *N*	Events *N*	Incidence rate (95% CI) Events/1000 person-years	Log-rank *P*-value	Hazard ratio (95%CI) Age adjusted	*P*-value	Multifactorial adjustment	*P*-value	Power
Women									
Wild type/wild type	3035	471	12 (11–13)	–	1.0	–	1.0	–	–
H63D/wild type	942	147	12 (10–14)	0.93	1.0 (0.8–1.2)	0.94	1.0 (0.9–1.2)	0.82	1.3
H63D/H63D	84	18	18 (11–28)	0.06	1.4 (0.9–2.2)	0.07	1.3 (0.8–2.1)	0.11	1.8
C282Y/wild type	401	46	8.8 (6.6–12)	0.12	0.7 (0.5–1.0)	0.10	0.8 (0.6–1.0)	0.15	2.0
C282Y/H63D	75	9	9.3 (4.5–18)	0.53	0.7 (0.4–1.4)	0.46	0.7 (0.4–1.4)	0.46	1.8
C282Y/C282Y	14	3	18 (5.9–56)	0.13	1.7 (0.5–5.2)	0.18	1.5 (0.5–4.8)	0.23	2.8
Total	4551	694							
Men									
Wild type/wild type	2493	412	13 (12–14)	–	1.0	–	1.0	–	–
H63D/wild type	765	140	15 (13–17)	0.23	1.1 (0.9–1.3)	0.35	1.1 (0.9–1.3)	0.32	1.4
H63D/H63D	60	12	16 (9–29)	0.45	1.2 (0.7–2.1)	0.58	1.3 (0.7–2.2)	0.43	2.0
C282Y/wild type	366	67	15 (12–18)	0.39	1.1 (0.9–1.4)	0.41	1.1 (0.7–1.5)	0.37	1.4
C282Y/H63D	43	7	13 (6–27)	0.98	1.0 (0.5–2.1)	0.99	1.0 (0.5–2.1)	0.97	2.1
C282Y/C282Y	7	3	38 (12–119)	0.02	3.5 (1.1–11)	0.02	3.7 (1.2–12)	0.01	3.7
Total	3734	641							
All									
Wild type/wild type	5528	883	13 (12–13)	–	1.0	–	1.0	–	–
H63D/wild type	1707	287	13 (12–15)	0.42	1.1 (0.9–1.2)	0.47	1.1 (0.9–1.2)	0.33	1.2
H63D/H63D	144	30	17 (12–25)	0.10	1.3 (0.9–1.8)	0.18	1.3 (0.9–1.8)	0.21	1.8
C282Y/wild type	767	113	12 (10–14)	0.40	0.9 (0.8–1.1)	0.42	0.9 (0.8–1.1)	0.49	1.3
C282Y/H63D	118	16	11 (6–17)	0.52	0.8 (0.5–1.3)	0.41	0.8 (0.5–1.4)	0.44	1.9
C282Y/C282Y	21	6	25 (11–55)	0.10	2.3 (1.0–5.1)	0.04	2.4 (1.1–5.3)	0.04	2.1
Total	8285	1335							

All participants were free of cancer at study entry.
Multifactorial adjustment was for age, gender (only 'All'), body mass index, tobacco consumption, smoking habits and alcohol consumption.
For women nulliparity, use of hormone replacement therapy and use of oral contraceptive drugs were also included.
Power: 80% power to detect the hazard ratio shown at two-sided $P = 0.05$.

individuals with C282Y/C282Y [30]. It has recently been demonstrated that hepcidin is a 'master regulator' of iron homeostasis [31]. This peptide hormone is crucial for modulating the amount of iron taken up from the diet and its distribution into various compartments of the body (erythrocytes, recycling, etc.). Patients with hereditary haemochromatosis have inappropriately low levels of this peptide regardless of which gene is mutated (*HFE*, *TFR2*, *HAMP*, *FPN*, etc.) [31]. Hepcidin is downregulated in the presence of oxidative stress [32].

There might, however, be different pathways leading to cancer in individuals with high iron stores in general and specifically in individuals with haemochromatosis genotype C282Y/C282Y, explaining the sex differences seen in our population-based study. Genetic haemochromatosis is not a sex-specific disease; however, there are sex differences. The clinical penetrance for haemochromatosis genotype C282Y/C282Y is well known to be higher in men than in women, simply because women bleed regularly before the menopause [33]. This is in accordance with our observation of a significant association between C282Y/C282Y genotype and increased cancer risk in men but with a similar trend in women. We speculate, that just after the menopause, in some women the sudden rise in serum iron concentration (because of

Table 4 *Risk of cancer subgroups according to transferrin saturation and hemochromatosis genotype*

	Multifactorially adjusted hazard ratio (95%CI)[a]											
	Transferrin saturation[b]						Hemochromatosis genotype[c]					
	Women		Men		All		Women		Men		All	
Endpoints	≥60% (IQR: 65–80%)	*P*	≥60% (IQR: 65–79%)	*P*	≥60% (IQR: 65–79%)	*P*	C282Y/ C282Y	*P*	C282Y/ C282Y	*P*	C282Y/ C282Y	*P*
Gastrointestinal cancer	3.0 (1.0–9.3)	0.08	1.1 (0.4–2.6)	0.91	1.4 (0.7–2.8)	0.35	No events	–	3.4 (0.5–25)	0.24	1.0 (0.1–6.9)	0.98
Liver cancer	9.6 (1.3–73)	0.02	4.4 (1.1–20)	0.04	5.9 (1.8–20)	0.004	No events	–	No events	–	No events	–
Female cancer	3.2 (1.3–7.7)	0.01	NA	–	Only women	–	1.1 (0.1–7.5)	0.99	NA	–	Only women	–
Male cancer	NA	–	1.5 (0.5–4.0)	0.44	Only men	–	NA	–	No events	–	Only men	–
Respiratory cancer	2.4 (0.6–9.8)	0.22	0.6 (0.2–1.9)	0.41	0.9 (0.4–2.1)	0.78	4.9 (0.4–19)	0.32	4.1 (0.6–30)	0.16	3.0 (0.7–12)	0.29
Other cancer	0.7 (0.1–4.9)	0.69	0.9 (0.3–2.4)	0.84	0.8 (0.3–2.0)	0.67	1.3 (0.2–9.1)	0.61	4.2 (0.6–30)	0.16	1.8 (0.5–7.4)	0.42

IQR, interquartile range; NA, not applicable.
There were too few endpoints to show reliable results for hematological and urological cancers.
[a]Multifactorial adjustment included: (i) Gastrointestinal and liver cancer: age, body mass index, tobacco consumption, smoking habits and alcohol consumption; (ii) Female cancer: age, body mass index, alcohol consumption, hormone replacement therapy, oral contraceptive drugs and nulliparity; (iii) Male cancer: age; (iv) Respiratory cancer: age, body mass index, tobacco consumption, smoking habits and alcohol consumption; (v) Other cancer: age.
[b]Reference category was <50% for women and men, respectively.
[c]Reference category was wild type/wild type for women and men, respectively.

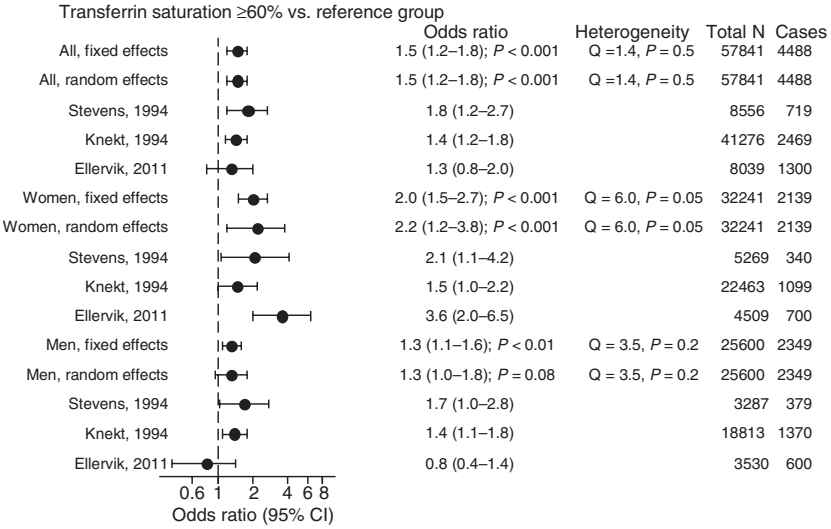

Fig. 2 *Meta-analysis of prospective studies of risk of any cancer (transferrin saturation ≥60% vs. reference group). The reference groups varied slightly across studies (≤30% to <60%) (see Supplementary Table S3). Horizontal lines indicate confidence intervals, and filled circles show the risk estimates.*

the lack of the physiological premenopausal blood loss) may mimic a mild iron poisoning with sudden and rapid oxidative stress on the liver resulting in downregulation of hepcidin [32] and leading to an uncontrolled iron overload. Moreover, there is evidence that more women (in any age group) than men take dietary supplements [34] including iron supplements, despite the fact that those taking supplements appear to be least likely to need them because of a more balanced diet [34]; thus, the postmenopausal iron overload may be even more exaggerated. However, the role of sex in the regulation of human hepcidin gene expression in the liver remains unclear [35]. Thus, the causes of iron overload – genetic, lifestyle or endogenous (menopause) – may be different between the two sexes, but the final pathway may be common, through oxidative stress and downregulation of hepcidin leading to unlimited iron influx to the plasma from duodenal cells. This may also explain the increased risk of liver cancer in both women and men with elevated levels of transferrin saturation. Furthermore, oxidative stress in women increases breast carcinogenesis [36].

Interaction of the *HFE* gene [3] with other genes may also be involved in cancer development. Evidence suggests that part of the short arm of chromosome 6p, especially the region 6p21–p23, harbours one or more oncogenes directly involved in tumour progression [37]; the *HFE* gene is located on 6p21.3 [3]. Also, other genetic modifiers such as HLA-A3 [38] and common variants in the genes of the hepcidin pathway [39] have been shown to modulate penetrance of HFE-associated haemochromatosis, leading to a more severe phenotypic expression of the *HFE* gene.

An association between elevated transferrin saturation and increased risk of any cancer in both women and men was found in the present meta-analysis. This finding is supported by results from a clinical trial of the effect of iron reduction [40] and from a cohort of regular blood donors [41] suggesting an effect of iron reduction on decreased risk of cancer.

An association between liver cancer and increased transferrin saturation is in accordance with the previous findings [9, 33]; however, although liver cancer is part of clinical haemochromatosis, we did not find any C282Y/C282Y homozygote with liver cancer, indicating the low penetrance of the genotypic disease compared with that of the biochemical disease, which again underscores the fact that risk of liver cancer according to C282Y/C282Y genotype estimated from case–control studies may be overestimated [5] because of ascertainment bias. However, owing to the low number of C282Y homozygous individuals, our study may not be adequately powered for this analysis.

Increased risk of cancer outside the liver was found in one cross-sectional study of patients with hereditary haemochromatosis [42]; however, risk of a variety of cancers in individuals with iron overload has been evaluated in various prospective [6–17] and case–control studies [18] with conflicting results, and C282Y/C282Y genotype was not found to be associ-

ated with cancer outside the liver in a recent meta-analysis of case–control studies [5].

We found that using a threshold of transferrin saturation of 50% or 60% seemed to be a better predictor than transferrin saturation quartiles for risk of any cancer, in agreement with the previous studies [10, 16]. One study, though, showed an increased risk of cirrhosis and liver cancer for transferrin saturation levels above 40% [9], a finding that could not be confirmed for risk of any cancer in our population-based study.

It remains unclear whether measurement of ferritin or transferrin saturation is the better predictor of hereditary haemochromatosis. Patients with hereditary haemochromatosis [43] with mild iron excess can have values of ferritin within the reference range; on the other hand, patients with increased transferrin saturation may never develop organ damage, and ferritin levels will detect the majority of patients who will be clinically affected. However, in the present study, measuring the future risk of cancer gives an elevated transferrin saturation earlier in life and supports a role for the measurement of transferrin saturation in the assessment of cancer risk.

Limitations include that we studied whites only, and therefore, our results may not necessarily apply to other ethnic groups. Although we included almost 9000 individuals, those with either elevated transferrin saturation levels or C282Y/C282Y genotype were relatively rare. Therefore, we cannot completely exclude the possibility that some of the results of the present study represent chance findings, and we would therefore like to see our results replicated in other studies. However, the fact that we observed increased cancer risk both for elevated transferrin saturation and C282Y/C282Y genotype as well as the fact that the meta-analysis confirmed our population-based results on risk of any cancer according to transferrin saturation is re-assuring. Nevertheless, as C282Y/C282Y would be even more uncommon in populations that do not have significant Celtic ancestry, the effectiveness of genotyping entire populations outside of Northern Europe would be limited. We were not able to study the effect of iron supplements or dietary iron intake, as these were not assessed as part of the CCHS. Ferritin concentrations, haemoglobin levels and reticulocyte counts were not available for the entire CCHS cohort; however, we have previously reported that individuals with C282Y/C282Y in the CCHS, matched 1 : 2 to individuals with the five other *HFE* genotypes, had significantly increased ferritin concentrations [44]. Furthermore, recent heavy alcohol use was not recorded, and transferrin saturation was a single nonfasting measurement that was not repeated to confirm an elevated value. Thus, we cannot exclude the possibility that some individuals with high transferrin saturation could have had bone marrow suppression, hepatocellular injury or a transient nonspecific rise in transferrin saturation, rather than increased iron stores. Therefore, and because individuals with C282Y/C282Y were referred to a hospital for management and possible treatment in 2001, our risk estimates for cancer may indeed be conservative.

Several high-throughput assays have been developed to test levels of hepcidin in the serum and urine[45], and these assays have a high correlation with whole-body iron loading and corresponding disease. As iron dysregulation is dependent on hepcidin in patients with hereditary haemochromatosis, it may be more useful to test for this peptide; measurement of hepcidin might allow clinicians to avoid the problems associated with measuring either ferritin or transferrin saturation alone. However, hepcidin measurements were not available to explore this possibility.

Early diagnosis of iron overload and instigation of appropriate treatment with repeated venesection can prevent the consequences of hereditary haemochromatosis and restore normal life expectancy [46]. If a causal association does indeed exist between iron overload and cancer risk, then patients with iron overload and C282Y/C282Y genotype may be candidates for individualized cancer screening programmes [42]. Targeted case finding for men of Northern European ancestry has previously been proposed [47], but it may be worth focusing also on women with iron overload, as they seem to be at a considerably increased risk of cancer.

In conclusion, we have observed that elevated transferrin saturation levels in women, and haemochromatosis genotype C282Y/C282Y in men, are associated with an increased risk of cancer. Therefore, our results support the implementation of cancer screening programmes in patients with iron overload or with C282Y/C282Y.

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Conflict of interest statement

No conflicts of interest to declare.

Author contribution

Christina Ellervik designed and conducted the research, analysed and interpreted the data, performed the statistical analysis and wrote the manuscript. Anne Tybjaerg-Hansen designed the research, collected the data and edited the manuscript. Børge G. Nordestgaard designed the research, collected, analysed and interpreted the data and edited the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Risk of any first cancer according to transferrin saturation in quartiles.

Table S2. Characteristics of individuals with C282Y/C282Y.

Table S3. Characteristics of prospective studies in systematic review.

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