Total and Cause-Specific Mortality by Elevated Transferrin Saturation and Hemochromatosis Genotype in Individuals With Diabetes: Two General Population Studies

Christina Ellervik,[1,2] Thomas Mandrup-Poulsen,[3,4] Anne Tybjærg-Hansen,[2,5] and Børge G. Nordestgaard[2,6]

OBJECTIVE

Mortality is increased in patients with hereditary hemochromatosis, in individuals from the general population with increased transferrin saturation (TS), and also in patients with type 1 diabetes and increased TS from a highly specialized diabetes clinic. Thus, we have recommended targeted screening for TS in specialized diabetes clinics. Whether mortality is also increased in individuals from the general population with diabetes and increased TS is unknown.

RESEARCH DESIGN AND METHODS

In two Danish population studies (*N* = 84,865), we examined mortality according to baseline levels of TS and hemochromatosis genotype (*HFE*) G → A substitution at nucleotide 845 in codon 282 (C282Y/C282Y) in individuals with diabetes (type 1, *N* = 118; type 2, *N* = 3,228; total, *N* = 3,346).

RESULTS

The cumulative survival rate was reduced in individuals with diabetes with TS ≥50% vs. <50% (log-rank; *P* < 0.0001), with median survival ages of 66 and 79 years, respectively. The hazard ratio (HR) for TS ≥50% vs. <50% was 2.0 (95% CI 1.3–2.8; *P* = 0.0004) for total mortality overall (and similar for men and women separately); 2.6 (1.3–5.4; *P* = 0.008) for neoplasms; and 3.4 (2.0–6.0; *P* = 0.00002) for endocrinological causes. A stepwise increased risk of total mortality was observed for stepwise increasing TS (log-rank test, *P* = 0.0001), with an HR for TS ≥70% vs. TS <20% of 4.8 (2.0–12; *P* = 0.0006). The HR for total mortality in individuals with diabetes for C282Y/C282Y versus wild type/wild type was 3.3 (1.04–10; *P* = 0.04), and for C282Y/C282Y and TS ≥50% versus wild type/wild type and TS <50% was 6.0 (1.5–24; *P* = 0.01). Six percent of these premature deaths can possibly be avoided by early screening for TS or *HFE* genotype.

CONCLUSIONS

Individuals with diabetes, ascertained in the general population, with increased TS or *HFE* genotype have a twofold to sixfold increased risk of premature death.

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[1]Department of Clinical Biochemistry, Næstved Hospital, Copenhagen University Hospital, Copenhagen, Denmark
[2]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[3]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[4]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden
[5]Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark
[6]Herlev Hospital, Copenhagen University Hospital, Copenhagen, Denmark

Corresponding author: Christina Ellervik, christina@ellervik.dk.

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Hereditary hemochromatosis is an autosomal-recessive disease, characterized by lifelong iron accumulation in various organs (e.g., the endocrine pancreas and the liver) (1). Eighty-three percent of hereditary hemochromatosis is explained by homozygosity for a G → A substitution at nucleotide 845 in codon 282, changing a cysteine to tyrosine (C282Y) in the hemochromatosis genotype (*HFE*) gene located at chromosome 6p21.3 (2). The protein HFE is a transmembrane glycoprotein (2) located at the basolateral membrane of crypt enterocytes that inhibits iron export into the circulation. Homozygosity for C282Y prevents formation of a disulfide bond in the protein and prevents cell-surface expression of the protein (3), leading to unregulated absorption of iron and iron overload (1).

There is evidence that *HFE* C282Y/C282Y (4,5), as well as iron overload independent of *HFE* genotype (6), both confer a risk of diabetes. There is also evidence for increased risk of premature death due to organ damage in patients with clinically overt hereditary hemochromatosis (7–11) in individuals from the general population with increased transferrin saturation (TS) (12), independent of *HFE* genotype (13), and in patients with diabetes and increased TS or *HFE* genotype from a highly specialized diabetes clinic, the Steno Diabetes Centre (Gentofte, Denmark) (14). In contrast, there is no evidence for increased risk of premature death in individuals with *HFE* genotype C282Y/C282Y in population-based studies (13,15–17) or in patients with type 2 diabetes who are of mixed ethnicity (18). Importantly, however, early detection and treatment of iron overload before the development of diabetes and cirrhosis can prevent excess mortality (10,19) and can restore normal life expectancy (7–10). Also, a recent study showed that patients with diabetes who are receiving maintenance hemodialysis with serum ferritin levels >700 ng/mL had a slightly increased risk of 1-year mortality (20).

A recent study in patients with hereditary hemochromatosis demonstrated a decline in diabetes prevalence in those patients who received a diagnosis after determining that they carried the *HFE* gene compared with those who received a diagnosis before such a determination (21), suggesting that awareness of hemochromatosis in general and development of diabetes in those patients in particular will translate into a greater life expectancy. Furthermore, we demonstrated a decline in mortality in patients with type 1 diabetes who were offered targeted screening for TS (14). Thus, we have recommended targeted screening for TS in specialized diabetes clinics (4,14).

Whether mortality is also increased in Caucasian individuals from the general population with diabetes and increased TS or *HFE* genotype C282Y/C282Y is unknown. If this was the case, however, a recommendation for targeted screening could also cover individuals with diabetes in the general population, when these individuals see their general practitioner for regular diabetes check-ups (i.e., if they have not already received a diagnosis hemochromatosis).

Therefore, in this study, we investigate total and cause-specific mortality according to increased TS or instances of *HFE* genotype C282Y/C282Y in Caucasian Danish individuals with diabetes from two general population studies.

RESEARCH DESIGN AND METHODS

Using two similar, but independent, Danish population-based follow-up studies conducted among Caucasian individuals, The Copenhagen General Population Study (CGPS; *N* = 2,971) 2003–2007 examination and The Copenhagen City Heart Study (CCHS; *N* = 375) 1991–1994 (12), we included 3,346 individuals with any diabetes from a total population size of 84,865 individuals. Information on prevalent diabetes was obtained from The National Danish Patient Registry (type 1 diabetes: ICD-8 [code 249], ICD-10 [code E10]; type 2 diabetes: ICD-8 [code 250], ICD-10 [codes E11, E13, and E14]; and from information on self-reported diabetes and antidiabetic medication. Individuals with undiagnosed diabetes but a nonfasting blood glucose >11 mmol/L were also included as having type 2 diabetes (*N* = 141). In total, 118 individuals had type 1 diabetes, and 3,228 individuals had type 2 diabetes. Individuals in the two studies were enrolled and examined similarly (12) with questionnaires and health examinations. The studies (KF-100.2039/91, KF-01-144/01, and H-KF-01-144/01) were approved by Herlev Hospital and Danish ethical committees. Written informed consent was obtained from all participants in both studies; there was no overlap of individuals between the two studies. The studies complied with the Declaration of Helsinki.

TS

TS (percentage) was determined as iron concentration (in micromoles per liter) divided by 2 × transferrin concentration (in micromoles per liter) × 100. Transferrin was measured by turbidimetry, and iron by colorimetry (Konelab, Helsinki, Finland). A threshold TS ≥50% was chosen as being suggestive of increased TS, in accordance with accepted clinical practice (22–24). To explore a graded relationship, TS was divided into the following seven categories: TS <20%; TS ≥20% but <30%; TS ≥ 30% but <40%; TS ≥40% but <50%; TS ≥50% but <60%; TS ≥60% but <70%; and TS ≥70%. The median TS was 22% (interquartile range [IQR] 17–28%; range 2–98%). All individuals with diabetes had TS level determined.

Genotyping

Genotyping of the CCHS for C282Y (single nucleotide polymorphism database number rs1800562), a G/A nucleotide change at position 845 in the *HFE* gene (2), and H63D (single nucleotide polymorphism database number rs1799945), a C/G nucleotide change at position 187 in the *HFE* gene (2), was performed by allele-specific amplification (25), and restriction enzyme digestion was used to confirm the genotyping (4). The amplification refractory mutation system simultaneously detects both *HFE* mutations C282Y and H63D, including sense and antisense primers for C282Y and H63D, and human growth hormone as an internal amplification control (25). Genotyping of the CGPS was performed by a TaqMan assay (Applied Biosystems, Foster City, CA) (14). *HFE* genotypes

were available in 1,865 individuals with diabetes.

Other Characteristics

Individuals were questioned about alcohol consumption, smoking habits, medication, and physical activity. Body mass index was calculated as weight in kilograms divided by squared height in meters. Plasma total cholesterol was measured enzymatically (26). Diabetic macrovascular and microvascular complications were not recorded in the health study; however, from The National Danish Patient Registry information on ischemic heart disease (IHD) (ICD8: 410–414, ICD10: I20–I25) and ischemic cerebrovascular disease (ICVD) (ICD8: 432–435, ICD10: I63–I64, G45) were available and from the health study levels of plasma-creatinin >90 mmol/L for women and >100 mmol/L for men as proxies for renal impairment) (27) were available.

End Points

Using the Central Person Registry Number, a number unique to every person living in Denmark, information on total and cause-specific mortality was obtained from the time of blood sampling through linkage to the Danish Civil Registration System (28) until 7 June 2011, and to the National Danish Causes of Death Registry (NDCDR) (29) until 31 December 2009, due to a delay in the updating of cause-specific death diagnoses in this registry. The NDCDR contains information on all underlying and contributing causes of death: until 2007, the coding was performed by the Danish National Board of Health using paper-based death certificates completed by physicians in hospitals, general practices, or forensic medicine departments (29); after 2007, the coding was performed by the physician who verifies the death and issues the electronic death certificate, and who also classifies the causes of death according to ICD coding (29). The NDCDR has consistently used ICD codes (since 1994, ICD-10 codes). The following ICD-10 codes were used for cause-specific deaths in this study: neoplasms, C00–D48 (*N* = 128); liver cancer, C220, C221, C223, and C229 (*N* = 6); endocrinological (endocrine, nutritional and metabolic diseases), E00–E90 (*N* = 58); and cardiovascular (diseases of the circulatory system) I00–I99 (*N* = 141). The follow-up in the CGPS was from 2003 to 2011 (through June 2011) (median 4 years [IQR 2–6 years]), whereas follow-up in the CCHS was from 1991 to 1994 (through June 2011) (median 10 years [IQR 5–17 years]). Follow-up information was acquired for all participants. During a median time period of 4 years of follow-up (maximum, 18 years), 541 individuals with diabetes died.

Statistical Analysis

Stata/SE version 11.0 statistical software package (StataCorp, College Station, TX) was used. Mann-Whitney *U* tests and Pearson χ^2 tests were used for continuous and categorical variables, respectively. Two-sided *P* values <0.05 were considered significant. A priori, we stratified the main analyses by gender, because penetrance of clinically manifest hemochromatosis differs markedly between the two genders (1). In explorative analysis, we also stratified participants into seven groups of TS levels, as described above.

Cumulative survival was plotted with the use of Kaplan-Meier curves as a function of age, and differences between TS or *HFE* genotype were examined by log-rank tests. Cox proportional hazards regression was used to estimate hazard ratios (HRs) with 95% CIs. The assumption of proportional hazards was tested with the use of Schoenfeld residuals, and no violations were observed. The interaction of TS or *HFE* genotype with risk factors on mortality was evaluated by including two-factor interaction terms, one at a time, in the multifactorial Cox regression model. No significant or clinically relevant interactions were observed.

Crude HRs included adjustment for age and gender. For TS, multifactorially adjusted HRs included age, gender, alcohol consumption (intake of seven or fewer drinks vs. more than seven drinks per week), smoking habits (current smoker vs. nonsmoker; smoking history: 0, 0 to ≤10, and >10 pack-years [1 pack-year is equivalent to smoking 20 cigarettes/day for 365 days/year]), leisure time physical activity (almost completely inactive, some activity, regular activity, regular hard physical training) (30), BMI (<25 vs. ≥25 kg/m^2), plasma cholesterol (<5 vs. ≥5 mmol/L), antihypertensive medication (yes vs. no), plasma creatinine level (women ≤ 90 and >90 mmol/L; men ≤100 and >100 mmol/L), and history of IHD/ICVD (yes/no). For *HFE* genotype, multifactorial adjustment included age, gender, smoking, cohort, leisure time physical activity, and plasma cholesterol level; alcohol consumption, BMI, antihypertensive medication, plasma creatinine, and history of IHD/ICVD were not significant confounders in these analyses. Genotype analyses were also adjusted for cohort, as genotype distributions differed between cohorts.

Population attributable risk was estimated as $[f(\mathrm{HR}-1)]/[1+f(\mathrm{HR}-1)]$, where *f* is the frequency of TS ≥50% in the population, and HR is the HR for total mortality (31).

For each analysis, we calculated the HR that could be detected with 80% power assuming a two-sided $P < 0.05$ using PASS software (NCSS, Kaysville, UT). Study power was too small to study type 1 diabetes alone, or to stratify results for sex- or cause-specific mortality for *HFE* genotype because only 11 individuals with diabetes had the C282Y/C282Y genotype. Likewise, it was not possible to present specific data for cause-specific mortality due to liver cancer, since only six individuals with diabetes died of liver cancer.

RESULTS

Table 1 lists the characteristics of participants at study entry. Those who died were more often men, were older at baseline, had received a diagnosis of diabetes at an older age, had a shorter diabetes duration, had higher number of pack-years of smoking tobacco, were more often current smokers, were more physically inactive, more often had a history of IHD/ICVD (only CGPS), and more often had elevated plasma creatinine levels compared with those who survived (Table 1). There were no differences in type of diabetes, BMI, plasma cholesterol, antihypertensive medication, or alcohol consumption.

TS

The cumulative survival was reduced in individuals with diabetes with TS ≥50%

Table 1—Baseline characteristics of participants with diabetes in two population-based follow-up studies

	CGPS		CCHS	
Characteristics	Alive (n = 2,715)	Dead (n = 256)	Alive (n = 90)	Dead (n = 285)
Women (%)	45	32*	50	36**
Age (years)	65 (58–72)	73 (67–79)*	58 (54–64)	70 (62–75)**
Recruitment dates	2003–2011		1991–1994	
Follow-up (years)	4 (2–6)		10 (5–17)	
Type 2 diabetes (%)	96	98	98	99
Type 1 diabetes (%)	4	2	2	1
Age at diabetes onset (years)	62 (53–70)	70 (62–77)*	61 (51–67)	69 (60–76)*
Diabetes duration (years)	5 (2–10)	4 (2–9)***	17 (12–20)	6 (2–14)*
BMI >25 kg/m^2 (%)	80	79	72	78
Tobacco consumption of >10 pack-years[1] (%)	38	48***	48	64***
Current smoker (%)	20	27***	39	44
Plasma cholesterol level >5 mmol/L (%)	44	39	88	84
Antihypertensive medication (%)	60	65	21	30
Alcohol consumption >84 g/week (>7 units/week) (%)	50	46	33	36
Physically inactive[2] (%)	11	20*	13	22**
IHD or ICVD (%)	16	32*	8	14
Elevated plasma creatinine level[3] (%)	11	30*	30	45**

Variables are expressed as the median (IQR) or proportion. Statistical comparisons were made using two-sided Mann-Whitney U test and Pearson χ^2 test, as appropriate. *$P < 0.001$. **$P < 0.05$. ***$P < 0.01$. [1]A pack-year is equivalent to smoking 20 cigarettes each day for 1 year. [2]Physical activity was leisure time physical activity (almost completely inactive, some activity, regular activity, regular hard physical training). [3]Women >90 μmol/L; men >100 μmol/L.

vs. <50% (log-rank test, $P < 0.0001$), and overall median survival time was 66 years (TS ≥50%) and 79 years (TS <50%). Crude HRs for total mortality for TS ≥50% vs. <50% were 2.0 (95% CI 1.3–2.8; $P = 0.0004$) overall, 1.8 (1.2–2.8; $P = 0.003$) in men, and 3.2 (1.2–8.8; $P = 0.02$) in women (Fig. 1); and 1.4 (0.9–2.2; $P = 0.1$) in CCHS and 2.6 (1.3–5.0; $P = 0.006$) in CGPS. Multifactorially adjusted analyses, analysis for type 2 diabetes only, and analysis excluding *HFE* genotypes (C282Y/C282Y and C282Y/H63D) showed similar results (Fig. 1). Crude HRs for cause-specific death due to neoplasms was 2.6 (1.3–5.4; $P = 0.008$), whereas death due to cardiovascular causes was not different by TS ≥50% vs. <50% (Fig. 1). Crude HRs for cause-specific death (Fig. 1) due endocrinological causes was 3.4 (2.0–6.0; $P = 0.00002$); thus, in individuals with diabetes and TS ≥50%, 26% had an endocrinological cause of death, whereas in individuals with TS <50%, only 5% had an endocrinological cause of death. The 163 registered individuals who died of endocrinological causes, all died of diabetes, but one also had primary adrenocortical insufficiency noted on the death certificate.

A stepwise increased risk of total mortality was observed for stepwise increasing TS (log-rank test, $P = 0.0001$) with the highest risk conferred by TS ≥70% vs. TS <20% with an HR of 4.8 (95% CI 2.0–12; $P = 0.0006$) overall (Fig. 2). Analyses in men and women separately (Fig. 2), multifactorially adjusted analyses (Fig. 2), and analysis for type 2 diabetes only or analysis excluding *HFE* genotypes C282Y/C282Y and C282Y/H63D showed similar results (the two latter analyses are not shown). The population-attributable risk of total mortality overall among individuals with diabetes was 2% for TS ≥50%.

HFE

The crude HR for total mortality in individuals with diabetes and *HFE* C282Y/C282Y versus wild type/wild type was 3.3 (95% CI 1.04–10; $P = 0.04$) overall (Fig. 3); results were similar in individuals with type 2 diabetes only. The crude HR for total mortality in individuals with C282Y/C282Y or TS ≥50% versus those with wild type/wild type and TS <50% was 2.1 (1.4–3.0; $P = 0.0009$). Also, the crude HRs for total mortality in individuals with C282Y/C282Y and TS <50%, wild type/wild type and TS ≥50%, and C282Y/C282Y and TS ≥50% versus wild type/wild type and TS <50% were 2.0 (0.3–14; $P = 0.5$), 2.0 (1.02–3.7; $P = 0.04$), and 6.0 (1.5–24; $P = 0.01$), respectively (trend $P = 0.003$; TS-genotype interaction $P = 0.05$). Multifactorially adjusted HRs showed similar results. HRs for *HFE* genotypes other than C282Y/C282Y were not significant (Supplementary Table 1). The population-attributable risk of total mortality overall among individuals with diabetes was 2% for C282Y/C282Y, 6% for C282Y/C282Y or TS ≥50%, and 2% for C282Y/C282Y and TS ≥50%.

CONCLUSIONS

In two homogeneous Caucasian Danish population-based follow-up studies comprising 84,865 individuals, we identified 3,346 individuals with prevalent diabetes, with the majority having type 2 diabetes. We showed that individuals with diabetes with the threshold TS ≥50% vs. <50% have an increased risk of premature death overall, and that individuals with

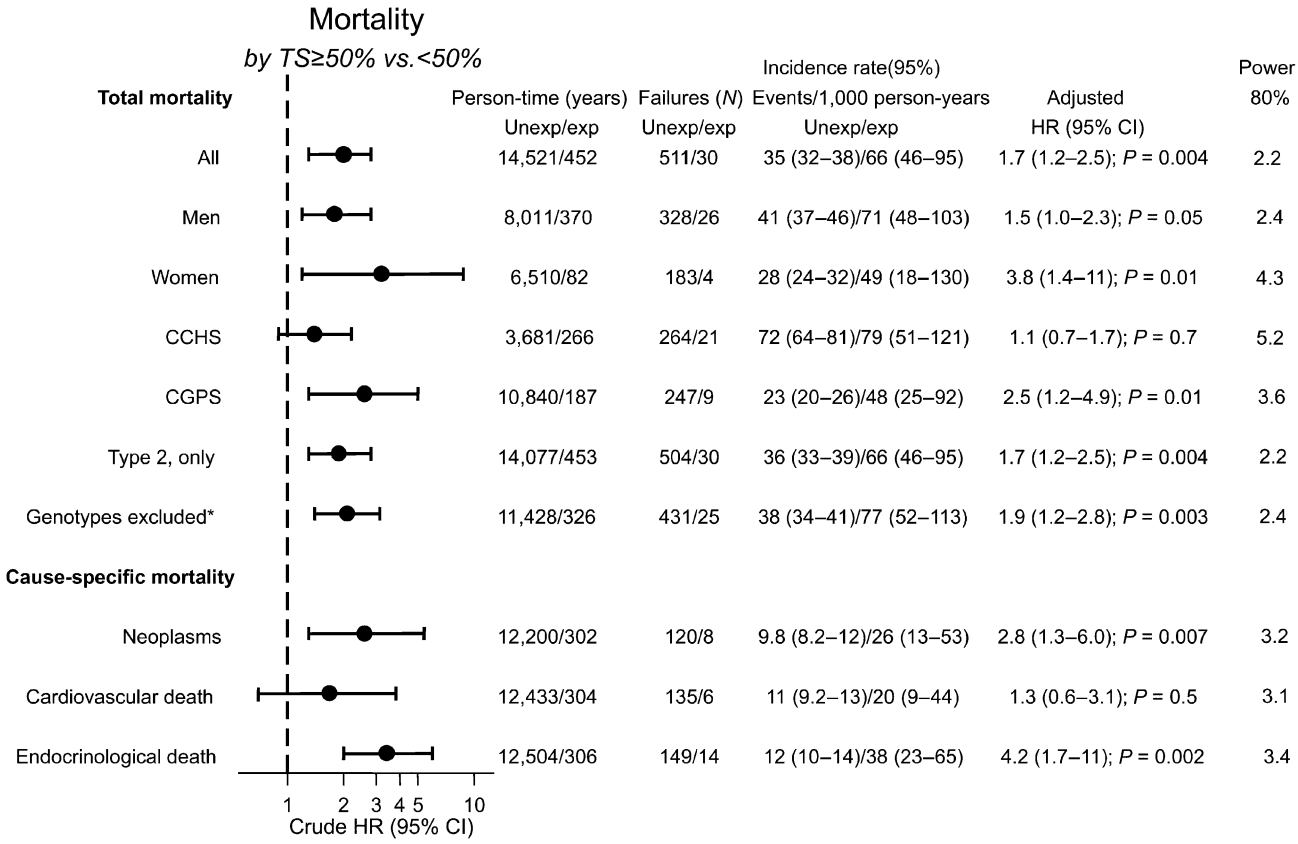

Figure 1—Total and cause-specific mortality by TS ≥50% vs. <50%. Power is 80% to detect a given HR. *Individuals with C282Y/C282Y and C282Y/H63D excluded from analyses. See text on statistics for adjustments. Exp, exposed.

diabetes had a stepwise increased risk of total mortality for stepwise increasing TS, with the highest risk conferred for TS ≥70%; results were similar in men and women, and for type 2 diabetes alone. Risk was independent of *HFE* genotype since risk was still increased when excluding *HFE* genotypes. Also, individuals with the threshold TS ≥50% vs. <50% had an increased risk of cause-specific death from neoplasms and endocrinological diseases, which were diabetes-related diagnoses. Moreover, elevated TS and C282Y/C282Y both increased the risk of premature death independently, but the joint effect of exposure to TS and C282Y/C282Y was higher than the sum of both effects. We calculated population-attributable risk showing that 6% of premature deaths among individuals with diabetes in the general population could potentially be avoided by early screening for TS or *HFE* genotype. Thus, TS and C282Y/C282Y independently and in combination increase the risk of premature death twofold to sixfold in individuals with diabetes from a general population study. These are novel findings.

Our findings that elevated TS increases the risk of premature death in individuals with type 2 diabetes underscore the results from our previous article (14) in patients with late-onset type 1 diabetes from a highly specialized diabetes clinic. Both of these studies were based on results of a baseline TS test at a random time point in life and not an early TS test. In the same recent article (14), we also showed that awareness of iron overload in a diabetes clinic with early measurement of TS may reduce mortality in patients with late-onset type 1 diabetes relative to that of the background diabetic population; furthermore, we showed that patients with type 2 diabetes who have an early measurement of TS have a mortality similar to the background diabetic population. However, for that patient cohort we lacked a control group who had a random TS test for comparison, which is provided in the current study.

Since the organ manifestations of iron overload include almost any organ (1), awareness of iron overload with early measurements of iron indices may increase survival. This is supported by a study of health check-ups and family screenings where subjects who were offered early detection of iron overload had improved survival compared with that of the background population (32). The improved survival in subjects who were offered an early TS test is likely conferred by the early diagnosis and treatment of iron overload or other conditions, but we have no data to demonstrate the reasons for specific health benefits of early measurement of TS. In our study, we did not measure ferritin, but another study (20) in patients with diabetes who were undergoing maintenance hemodialysis with serum ferritin levels >700 ng/mL had slightly increased 1-year mortality. Whether the biochemical testing should

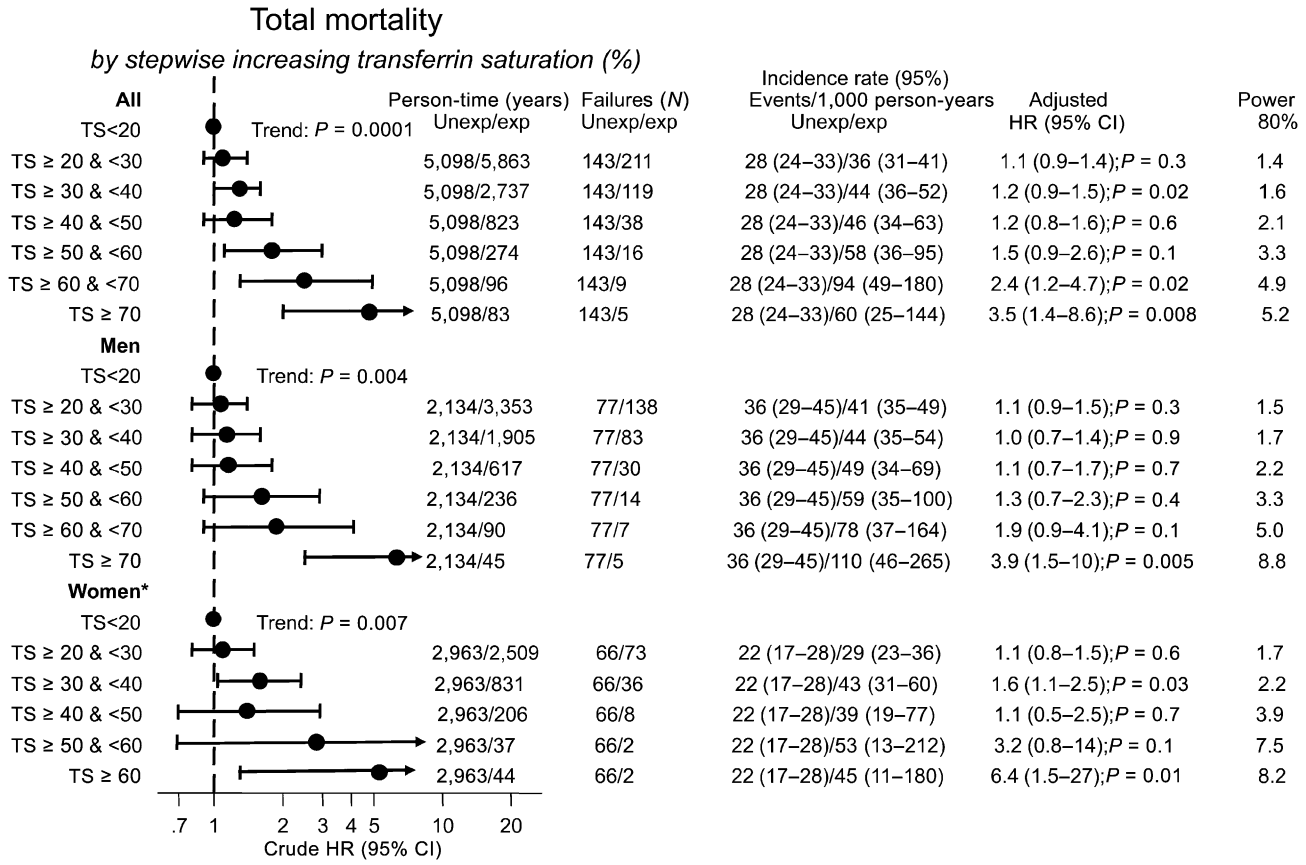

Figure 2—Total mortality by stepwise increasing levels of TS. Power is 80% to detect a given HR. See text on statistics for adjustments. *There were no deaths among women with TS ≥70%. Exp, exposed.

be for TS, ferritin, or both needs to be resolved (33). The dose-response relationship with increased risk of total mortality for stepwise increasing TS has also been shown in two previous population-based studies (12); however, risk in the population cohorts increased from TS ≥40%, whereas in this study, based on individuals with diabetes, risk increased from TS ≥30%. Thus, it could be speculated that in individuals with diabetes the TS cutoff should be even lower than 50%. To support this, it has been shown that iron depletion in patients with diabetes ameliorates HbA_{1c} levels, insulin secretion, insulin resistance (34), and vascular dysfunction (35).

In this study of individuals with mainly type 2 diabetes and in a previous study of patients with late-onset type 1 diabetes (14), we have shown that *HFE* genotype C282Y/C282Y alone or combined with elevated TS increases the risk of premature death; this is in accordance with the fact that patients with iron overload and manifest organ dysfunction have increased mortality (8–10). In population-based studies, C282Y/C282Y confers a risk of diabetes (4,5) but not a risk of premature death (13,15,17). Thus, it could be speculated that the development of organ dysfunction is needed before the genotypic effect confers the risk of premature death; however, the joint effect of *HFE* genotype with TS or ferritin level has not been studied in population-based mortality studies of C282Y/C282Y.

Our study results contrast with those of another Australian study (18) that could not show evidence of increased mortality related to iron overload or *HFE* genotype in patients with type 2 diabetes; however, that study had only 1,265 patients of mixed ethnicity, and had a shorter follow-up time and less power, and thus was not comparable to our study, which was larger and had patients of homogeneous ethnicity. It has previously been shown that ethnicity matters in terms of the risk conferred by iron overload and *HFE* genotype (5).

Remarkably, those who died in the study had received a diagnosis of diabetes later in life than those who survived, but they also had shorter diabetes duration; thus, this group of individuals may be susceptible to factors increasing their sensitivity to the detrimental effects of diabetes or may have had undiscovered and thus untreated diabetes for a longer time than those who had received a diagnosis earlier.

We did not have sufficient power to exclude a modestly increased risk of total mortality in the CCHS alone or of cardiovascular disease overall conferred by elevated TS; furthermore, we did not have sufficient power to exclude a modestly increased risk of total mortality conferred by other *HFE* genotypes than C282Y/C282Y; however,

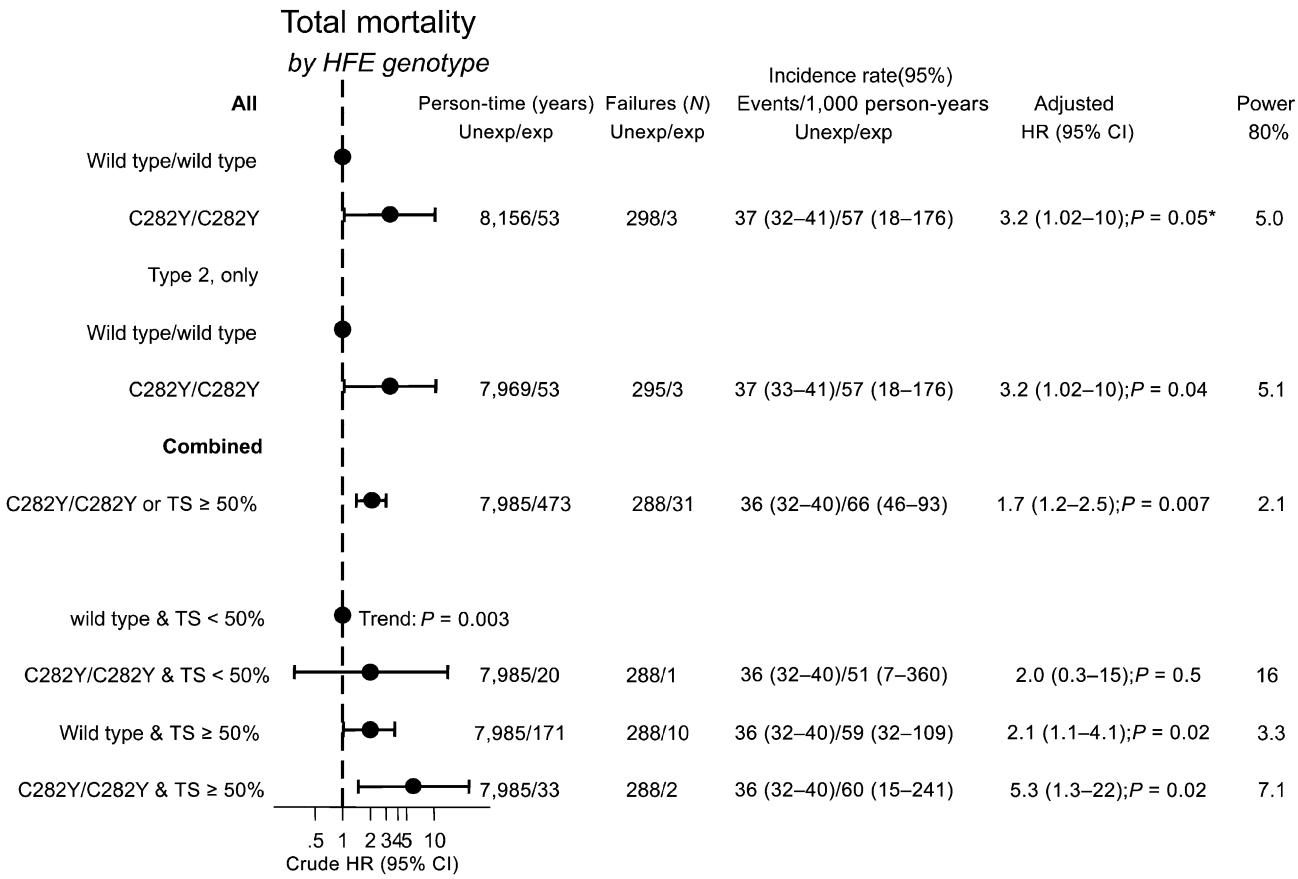

Figure 3—Total mortality by *HFE* C282Y/C282Y. Power is 80% to detect a given HR. *$P = 0.046$. See text on statistics for adjustments. Exp, exposed.

these genotypes have not previously been associated with increased mortality in individuals with diabetes (14).

The correctness of the underlying and contributing causes of death relies on the codes and on the physicians who have filled in the death certificates (29). Before 2007, it was the National Board of Health in Denmark who interpreted the written information on underlying and contributing causes of death on paper-based death certificates issued by physicians, and translated this information to ICD codes; this practice may have resulted in misinterpretations (29). After 2007, electronic coding was performed by the physician who issues the electronic death certificate, and thus the coding relies on the diagnostic accuracy of the attending physicians (29). However, both systems share some general limitations. Differences in the causes of death may be due to new diagnostic techniques, increased focus on special diseases, and less focus on ill-defined diseases (29). Furthermore, in 1990 the legislation on autopsies in persons who died a natural death in Denmark was changed from a practice where a previous consent from the person who died or a consent from the family was not needed, to a practice where either of these consents was required. Thus, autopsy rates in Denmark have since declined and are low (<10%) (29). A recent meta-analysis (36) of the discrepancy between clinical and autopsy diagnoses estimated that 30% of the diagnoses listed on the death certificates are incorrect. Furthermore, in Denmark the causes of death are not regularly validated (29), as opposed to, for example, Finland where a validation report estimated that of 7% questionable death certificates, half were reassigned to a different ICD code (37). Thus, the correctness of the causes of death is crucial for mortality statistics and health surveillance but also for research purposes. However, total mortality in Denmark is based on the Danish Civil Registration System, which updates vital status continuously and is thus considered complete for Danish residents (28); however, for those persons who have emigrated or disappeared, death is only registered if the Danish authorities are informed about their death or the death occurred in Denmark (28).

The pathogenetic link between iron overload and increased mortality may be exerted through iron-catalyzed formation of hydroxyl radicals via the Fenton reaction and ensuing tissue oxidative stress (38). A recent study (39) showed increased mortality in patients with diabetes with C282Y/C282Y or iron overload and elevated urinary excretion of oxidized RNA. Thus, oxidative stress is linked to decreased survival.

This study is a genetic epidemiological association study of markers of mortality, and as such does not provide

proof of causality as would have been possible in an intervention study. Two recent articles (40,41) have reviewed the principles of screening according to the World Health Organization guidelines in the context of hereditary hemochromatosis and conclude that generalized population screening in primary care is generally not recommended; however, there may be a role for focused screening in Caucasian men (40) because 84% of men >55 years of age have elevated ferritin levels and 37% have ferritin levels >1,000 μg/L, which is a generally accepted threshold for organ impairment (41). The reasons for the conclusions, among others, are that the gold standard measure for the screening has not yet been clarified (TS, ferritin, or both, and/or genetic screening?); that the biological variability of TS is high; that the biochemically measured iron overload (in the case of ferritin overload) may have many other causes (42) that do not justify phlebotomy intervention (40); and that penetrance of homozygosity for C282Y/C282Y is low (40).

In a study of patients with late-onset type 1 diabetes (4), the positive and negative predictive values of TS ≥50% for detecting C282Y/C282Y were 26% and 100%, respectively; and the sensitivity and specificity were 100% and 96%, respectively. For comparison, in the current study among patients with type 2 diabetes, the positive and negative predictive values of TS ≥50% for detecting C282Y/C282Y were 12% and 99%, respectively; and the sensitivity and specificity were 64% and 97%, respectively. Likewise, in the general population from which the patients with diabetes in this study were identified, the positive and negative predictive values of TS ≥50% for detecting C282Y/C282Y were 12% and 99%, respectively; and the sensitivity and specificity were 70% and 99%, respectively. Thus, the specificities were comparable and high, and corresponded to those reported in The Hemochromatosis and Iron Overload Screening Study (HEIRS) (40). The sensitivity was highest among patients with late-onset type 1 diabetes; but in the general population overall and in patients with type 2 diabetes, sensitivities corresponded to those in HEIRS but were relatively low, limiting the role of TS as a screening test. The positive predictive values were low, as in HEIRS (40); thus, TS ≥50% reflects more than just homozygosity for C282Y/C282Y.

In current clinical practice, targeted screening for iron overload or *HFE* genotype has mainly been offered to patient populations in whom organ manifestations of iron overload have already developed. However, the results from the current study together with other survival studies (8,10,12,14,20,21,32,43) add up to the conclusion that with manifest organ disease the prognosis of survival is low. Thus, early detection of iron overload before organ manifestations is desirable in the future. Prerequisites are awareness among physicians and decision makers in health politics. However, cost-effectiveness analyses on screening still only recommend targeted screening and not population screening for hemochromatosis (44,45).

In summary, individuals with diabetes from the general population who had increased TS or *HFE* genotype had a twofold to sixfold increased risk of premature death.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. C.E. designed the study, analyzed and interpreted data, wrote and edited the manuscript, and constructed the figures and tables. T.M.-P. designed the study, interpreted data, and edited the manuscript. A.T.-H. and B.G.N. designed the study, contributed to data collection, interpreted data, and edited the manuscript. C.E. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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