Original Article

Journal of INTERNAL MEDICINE

doi: 10.1111/j.1365-2796.2010.02217.x

Haemochromatosis genotype and iron overload: association with hypertension and left ventricular hypertrophy

C. Ellervik[1,2], A. Tybjærg-Hansen[1,3,4], M. Appleyard[1,4], H. Ibsen[5,6] & B. G. Nordestgaard[1,4,6]

From the [1]Department of Clinical Biochemistry and the Copenhagen General Population Study, Herlev Hospital, Copenhagen University Hospital, University of Copenhagen, Herlev; [2]Department of Clinical Biochemistry, Næstved Hospital, University of Copenhagen, Naestved; [3]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, University of Copenhagen, Copenhagen East; [4]The Copenhagen City Heart Study, Bispebjerg Hospital, Copenhagen University Hospital, University of Copenhagen, Bispebjerg; [5]Department of Cardiology, Holbaek Hospital, Holbaek; and [6]LIFEGEN Study, Herlev Hospital, Copenhagen University Hospital, Naestved, Denmark

Abstract. Ellervik C, Tybjærg-Hansen A, Appleyard M, Ibsen H, Nordestgaard BG (Herlev Hospital, Copenhagen University Hospital, University of Copenhagen, Herlev; Næstved Hospital, University of Copenhagen, Naestved; Copenhagen University Hospital, University of Copenhagen, Copenhagen East; Bispebjerg Hospital, Copenhagen University Hospital, University of Copenhagen, Bispebjerg; and Holbaek Hospital, Holbaek; Denmark). Haemochromatosis genotype and iron overload: association with hypertension and left ventricular hypertrophy. *J Intern Med* 2010; **268**: 252–264.

Objective. We hypothesized that there is an association between haemochromatosis genotype C282Y/C282Y and/or iron overload and risk of hypertension and/or left ventricular hypertrophy (LVH).

Methods. We analysed data from a cross-sectional study of the general population including 8992 individuals from the Copenhagen City Heart Study (CCHS), a follow-up study of 36 480 individuals from the Copenhagen General Population Study (CGPS), and a case-only study of 3815 Scandinavians from the Losartan Intervention For End-point Reduction in Hypertension Genetic Substudy (LIFEGEN) with LVH and hypertension.

Results. In the CCHS, individuals with C282Y/C282Y versus wild type/wild type had an odds ratio for antihypertensive medication use of 4.8 (1.8–13; $P = 0.003$). In the CGPS, the corresponding hazard ratio was 1.7 (1.0–2.3; $P = 0.003$). Also, hazard ratios for antihypertensive medication use in the CGPS were 1.6 (1.0–2.6; $P = 0.05$) for transferrin saturation ≥80% vs. <50%, and 2.3 (1.3–4.2; $P = 0.005$) for C282Y/C282Y + transferrin saturation ≥80% vs. wild type/wild type + transferrin saturation <50%. These results were most pronounced in men above 55 years of age. We did not find any association between C282Y/C282Y or iron overload and LVH or hypertension (measured as blood pressure at a single occasion or continuous blood pressure), or LVH with hypertension in the CCHS or with severity of LVH in LIFEGEN.

Conclusions. We found that haemochromatosis genotype C282Y/C282Y and extremely elevated transferrin saturation either separately or combined were associated with increased risk of antihypertensive medication use. Therefore, testing for haemochromatosis genotype C282Y/C282Y and extreme transferrin saturation could be considered in patients with essential hypertension.

Keywords: haemochromatosis, HFE, hypertension, iron overload, left ventricular hypertrophy, transferrin saturation.

Introduction

Hereditary haemochromatosis is an autosomal recessive disorder characterized by iron overload and lifelong accumulation of iron primarily in the liver, pancreas and heart, leading to liver cirrhosis, diabetes and cardiomyopathy [1, 2]. Homozygosity for C282Y in the HFE gene is responsible for 83% of hereditary haemochromatosis in white individuals [3]. The earliest detectable biochemical anomaly is increased transferrin saturation [1], which generally represents an increased intestinal iron absorption; this may be followed by an increase in ferritin concentration, high enough to be suggestive of organ damage [1].

Increased thickness of the ventricular wall is probably the first, and still reversible, cardiac alteration due to iron deposition in the myocardium [4]. Later,

with increasing iron overload, left ventricular function becomes impaired and dilated cardiomyopathy develops [4]. The echocardiographic features of haemochromatosis are varying degrees of cardiomyopathy and left ventricular hypertrophy (LVH) [5], the latter of which is also a feature of hypertension [6].

Meta-analyses [7–9] did not show any association between HFE gene mutations or iron overload and ischaemic heart disease or myocardial infarction; however, the association between HFE gene mutations and diabetic microangiopathy is still controversial [10–13]. Also, accumulating evidence suggests that oxidative stress may alter the modulation of vascular tone [14], thereby affecting blood pressure leading to hypertension. Oxidation of iron generates free hydroxyl radicals through the Fenton reaction, producing oxidative stress in cells [15]. An increase in ferritin levels in men with essential hypertension has been shown [16]. Furthermore, it has been shown that arterial wall thickness was increased before the onset of cardiovascular complications in haemochromatosis patients and that this alteration was reversed by iron depletion [17]. It is therefore possible that individuals with haemochromatosis genotypes and/or iron overload are overrepresented amongst patients with hypertension and/or LVH.

We tested the hypothesis that the haemochromatosis genotype C282Y/C282Y and/or extreme elevation in transferrin saturation, which signals iron overload, are associated with hypertension and/or LVH. For this purpose, we used the data from three studies. (i) The cross-sectional Copenhagen City Heart Study (CCHS), which included 8992 individuals from the general population of whom, 1046 were on antihypertensive medication and 744 had LVH. (ii) A follow-up study of 36 480 individuals from the Copenhagen General Population Study (CGPS), of whom 7080 received antihypertensive medication. (iii) The Losartan Intervention For End-point Reduction in Hypertension Genetic Substudy (LIFEGEN), which included 3815 Scandinavian participants all with hypertension and LVH.

Materials and methods

Participants

The three studies were approved by the appropriate institutional review boards and ethical committees. Written informed consent was obtained from participants in all studies. This investigation conforms to the principles of the Declaration of Helsinki.

The Copenhagen City Heart Study

The CCHS is a study of the Danish general population initiated between 1976 and 1978 [18, 19]. We determined the genotype of 8992 white individuals of Danish descent from the 1991 to 1994 examination for C282Y and H63D [20, 21]. Clinically overt haemochromatosis was not diagnosed in any of the 23 C282Y/C282Y individuals before the study. Four of the 23 C282Y/C282Y individuals had been blood donors [21]. All living C282Y/C282Y individuals were informed of their genotype status in 2001. They were also informed of the possibility of a slightly increased risk of developing haemochromatosis, but that therapeutic phlebotomy could be considered. Of the 20 C282Y/C282Y individuals still alive in 2001, all were then referred to a hospital for possible treatment for haemochromatosis. Whether or not C282Y/C282Y individuals were treated with phlebotomy from 2001 and onwards have not been recorded.

The Copenhagen General Population Study

The CGPS is a study of the Danish general population initiated in 2003. We determined the genotype of 36 480 white individuals of Danish descent for C282Y and H63D. No subjects took part in both the CCHS and CGPS. Because clinically overt haemochromatosis was not diagnosed in any of the C282Y/C282Y individuals in the CCHS, those with C282Y/C282Yin the CGPS were not informed of their genotype status. We do not know whether any of these C282Y/C282Y individuals had a previous diagnosis of haemochromatosis.

Losartan Intervention For End-point Reduction in Hypertension Genetic Substudy

Losartan Intervention For End-point Reduction in Hypertension Genetic Substudy included 3815 Scandinavian patients with hypertension and electrocardiogram (ECG)-verified LVH [22–24] who were all genotyped for C282Y and H63D. All patients were white, and were recruited between June 1995 and April 1997 in Denmark (N = 904), Finland (N = 1262), Norway (N = 641) and Sweden (N = 1008).

Hypertension

CCHS and CGPS Patient-reported intake of antihypertensive medication was taken as the best indicator of hypertension in these studies, because it is likely to indicate that the participant's general practitioner found evidence of hypertension during several con-

sultations. Only in the CGPS were data available on the prescription date of antihypertensive medication. Blood pressure was measured by trained technicians using the London School of Hygiene sphygmomanometer on the left arm after 5 min rest with the subject in the sitting position. The disappearance of the Korotkoff sound (phase V) was used to determine diastolic pressure. The fall of the mercury column was set to 2 mm s^{-1}. The blood pressure cuff was 12 × 26 cm; however, a cuff that measured 15 × 38 cm was used for subjects with an upper arm circumference of >46 cm [25–27].

LIFEGEN Hypertension was defined as mean sitting diastolic blood pressure reading of 95–115 mmHg or mean sitting systolic blood pressure readings of 160–200 mmHg, after 1 and 2 weeks of single-blind placebo treatment [22–24].

Left ventricular hypertrophy

CCHS Left ventricular hypertrophy was diagnosed by standard 12-lead ECGs and coded by a core laboratory, according to the Minnesota code [28]: 3.1 and 3.3 with an R amplitude >26 mm in either of leads V5 or V6, an R amplitude >20 mm in any of leads I, II, III, aVF, an R amplitude >12 mm in lead aVL, an R amplitude of 15–20 mm in lead I, or an R amplitude in V5 or V6 plus S amplitude in V1 > 35 mm. Eligible ECG data were available for 8937 individuals.

LIFEGEN Left ventricular hypertrophy was diagnosed by standard 12-lead ECG by a core laboratory, and was defined according to criteria based on the product of Cornell voltage (RaVL + SV3) and QRS duration [22]: >2440 mm × ms in men, and the same product plus 6 mm in women [22]. Furthermore, Sokolow-Lyon voltage combination (SV1 + RV5 or RV6) >38 mm was accepted as an alternative criterion for LVH in both men and women.

Genotyping

Genotyping of the CCHS participants for C282Y (dbSNP: rs1800562), a G/A nucleotide change at position 845 in the HFE gene [3], and H63D (dbSNP: rs1799945), a C/G nucleotide change at position 187 in the HFE gene [3], by allele specific amplification [29], with restriction enzyme digestion to confirm genotyping [3, 20]. The amplification refractory mutation system (ARMS) simultaneously detects both hereditary haemochromatosis mutations C282Y and H63D, including sense and antisense primers for C282Y, H63D and human growth hormone as an internal amplification control [29]. Genotyping of the CGPS and LIFEGEN participants by a TaqMan assay (Applied Biosystems, Foster City, CA, USA) (details available from authors), and confirmed using sequencing. CCHS, CGPS and LIFEGEN populations were in Hardy–Weinberg equilibrium for C282Y and H63D.

Iron overload

CCHS and CGPS Iron overload was defined as transferrin saturation levels of ≥50%. Transferrin saturation (%) was determined as iron levels (in μmol L^{-1}) divided by 2× transferrin levels (in μmol L^{-1}) × 100. Transferrin was measured by turbidimetry and iron levels by absorption photometry (Konelab autoanalyzer; ThermoFisher Scientific, Waltham, MA, USA). Transferrin saturation data were available for 8201 individuals in the CCHS and 36 463 in the CGPS. Transferrin saturation was dichotomized in the CCHS (<50%, ≥50%) due to reduced power compared to the CGPS, in which transferrin saturation was divided into five groups (<50%, 50% to <60%, 60% to <70%, 70% to <80%, ≥80%).

Other characteristics

CCHS, CGPS and LIFEGEN Individuals were questioned about alcohol intake and smoking habit. Body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared. The presence of diabetes mellitus was defined as self-reported disease, treatment for diabetes and/or nonfasting plasma glucose >11 mmol L^{-1}. Plasma total cholesterol, HDL cholesterol and glucose levels were measured by colorimetric assays (Boehringer Mann-heim GmbH, Mannheim, Germany) in the study laboratory (CCHS, CGPS) [18, 19] or in a central laboratory (LIFEGEN) [22–24]. Risk factor status was determined at study entry for the CCHS and CGPS, and prior to randomization for LIFEGEN.

Study designs

CCHS Data from the CCHS were used in a cross-sectional design for these analyses; however, the total number of individuals available for the different analyses varies because of the number of individuals with different covariates or available end-points. We studied the association between: (i) genotype and iron overload; (ii) genotype or iron overload and risk of hypertension as (a) intake of antihypertensive medication, (b) hypertension based on a single blood pressure measurement with cut-off of 140/90 mmHg

and inclusion of those receiving antihypertensive treatment in the hypertensive group before analysis and (c) continuous blood pressure measurement [diastolic blood pressure, systolic blood pressure, pulse pressure (the difference between systolic and diastolic blood pressures)]; and (iii) genotype or iron overload and risk of LVH.

CGPS Individuals from the CGPS were followed from birth until the date of prescription of antihypertensive medication or end of follow-up at the enrollment date (i.e., 2 065 021 person-years).

LIFEGEN We performed a case-only study within LIFEGEN of (i) the variation in diastolic blood pressure, systolic blood pressure, pulse pressure, Cornell voltage–duration product (mm × ms) and Sokolow-Lyon voltage (mm) according to haemochromatosis genotype, and (ii) the severity of LVH according to haemochromatosis genotype using dichotomized values [above versus below median Cornell voltage product (2668 mm × ms) and Sokolow-Lyon voltage (29 mm)].

Statistics

NCSS-PASS software was used for power calculations. The free internet source Haploview from http://www.broadinstitute.org was used to estimate pairwise linkage disequilibrium. Otherwise, the statistical software package Stata/SE 9.0 was used. Two-sided probability values of $P < 0.05$ were considered significant. We used Bonferroni correction for multiple comparisons for genotype results, as we had five genotype comparisons for each end-point ($P \leq 0.01$ was considered significant).

For differences in continuous measurements (transferrin saturation, diastolic blood pressure, systolic blood pressure, pulse pressure, Cornell voltage-duration product and Sokolow-Lyon voltage) in the CCHS, CGPS or LIFEGEN according to haemochromatosis genotype or transferrin saturation, we used Mann–Whitney *U*-test for two-group comparisons and Kruskal–Wallis test for multiple comparisons. Correlation between transferrin saturation (logarithmically transformed) and continuous blood pressure measurement in the CCHS was examined with linear regression. For participants who were taking antihypertensive medication, we added 10 mmHg to the observed systolic blood pressure values and 5 mmHg to the observed diastolic blood pressure values [30, 31] in the analyses of blood pressure as continuous traits.

In the CCHS, logistic regression analyses estimating odds ratios for antihypertensive medication, hypertension and LVH according to haemochromatosis genotypes or iron overload were performed crude or adjusted.

In the CGPS, plots of cumulative incidences of treatment with antihypertensive medication with the use of Kaplan–Meier curves as a function of age were determined. Differences between haemochromatosis genotypes or degree of iron overload were tested for significance with the log-rank test. Cox proportional hazards regression (adjusted for age and gender) was used to estimate hazard ratios with 95% confidence intervals for risk of treatment with antihypertensive medication.

In LIFEGEN, logistic regression analyses estimating odds ratios for severity of LVH by either Cornell voltage product or Sokolow-Lyon voltage according to haemochromatosis genotype were performed crude or adjusted.

Adjustments were made for age, gender, BMI, smoking, alcohol consumption, cholesterol and HDL cholesterol levels and diabetes mellitus as shown in Table 1. To test for bivariate multiplicative interaction between genotype or iron overload and the covariates listed in Table 1 on end-points, two-factor interaction terms were included individually in the different models and tested for significance with a likelihood ratio test. No statistically significant interactions were observed. In analyses, iron overload was stratified for alcohol intake as excessive intake may increase transferrin saturation levels [32]; however, this did not affect the results.

We estimated the pairwise linkage disequilibrium, a measure of deviation from random association (i.e., no recombination), between the A allele of C282Y and the G allele of H63D. The measure r^2 represents the statistical correlation between two sites, and takes the value of 1 if only two haplotypes are present [33].

Absolute risks for antihypertensive medication by genotype or iron overload (transferrin saturation) were estimated by using the regression coefficients from a Poisson regression model. Absolute risks are presented as estimated incidence rates (events/10 years) in percentage. Smoking (no/yes) was entered as a covariate, but did not affect the results; therefore, data are shown without smoking as a covariate.

Table 1 *Characteristics of participants*

	CCHS	CGPS	LIFEGEN
Numbers (*N*)	8992	36 480	3815
Age (years)	60 (47–70)	58 (48–67)	66 (60–72)
Men (%)	44	46	46
Diastolic blood pressure (mmHg)	84 (75–92)	83 (75–90)	99 (94–104)
Systolic blood pressure (mmHg)	136 (122–152)	140 (126–155)	174 (165–185)
Pulse pressure (mmHg)	51 (42–65)	55 (45–68)	76 (66–87)
BMI (kg m^{-2})			
1st tertile	22 (20–23)	22 (21–23)	24 (23–25)
2nd tertile	25 (24–26)	26 (25–27)	27 (26–28)
3rd tertile	30 (28–32)	30 (29–32)	32 (31–34)
Smokers (%)	49	23	16
Alcohol consumption (units[a] $week^{-1}$)			
None (%)	21	14	40
1–4 (%)	23	20	43
5–7 (%)	14	14	9
8–10 (%)	11	12	4
More than 10 (%)	31	40	4
Cholesterol (mmol L^{-1})			
1st tertile	4.9 (4.5–5.3)	4.7 (4.3–5.0)	5.1 (4.7–5.4)
2nd tertile	6.1 (5.8–6.4)	5.7 (5.5–5.9)	6.1 (5.9–6.4)
3rd tertile	7.4 (7.0–8.0)	6.7 (6.4–7.2)	7.2 (6.9–7.8)
HDL cholesterol (mmol L^{-1})			
1st tertile	1.1 (1.0–1.2)	1.2 (1.0–1.3)	1.1 (1.0–1.2)
2nd tertile	1.5 (1.4–1.5)	1.6 (1.5–1.7)	1.5 (1.4–1.6)
3rd tertile	2.0 (1.9–2.3)	2.2 (2.0–2.4)	2.0 (1.8–2.2)
Diabetes mellitus (%)	4	4	11

[a]One unit of alcohol is equivalent to 12 g.
Values are frequencies (%) or medians (interquartile ranges).
BMI, body mass index; CCHS, Copenhagen City Heart Study; CGPS, Copenhagen General Population Study; HDL, high-density lipoprotein; LIFEGEN, Losartan Intervention For End-point Reduction in Hypertension Genetic Substudy; LVH, left ventricular hypertrophy.

Population-attributable risk was calculated as $[f(HR - 1)]/[1 + f(HR-1)]$, where f is the frequency of the genotype or transferrin saturation level of interest in the population and HR is the hazard ratio for antihypertensive medication.

Results

Characteristics of participants in the CCHS, CGPS and LIFEGEN are shown in Table 1. Minor allele frequencies in the three studies were 5.9%, 5.9% and 5.3%, respectively, for the A allele of C282Y and 13% in all studies for the G allele of H63D. These two alleles were not in linkage disequilibrium in the general population studies CCHS and CGPS; r^2 was 0.009 in both.

Genotype and iron overload

CCHS Amongst 8201 individuals in this general population, transferrin saturation levels were elevated in C282Y/C282Y versus wild type/wild type (Kruskal–Wallis test, $P = 0.0001$; Mann–Whitney U-test for individual comparisons against wild type/wild type, $P < 0.0001$) (data not shown).

Genotype and hypertension

CCHS We took use of antihypertensive drugs in 1046 of 8908 CCHS participants as the best indicator of hypertension in this study. Individuals with C282Y/C282Y had an increased risk of treatment with antihypertensive medication compared with wild type/wild type, with an adjusted odds ratio of 4.8 (1.8–13; $P = 0.003$) in all participants (Figure S1A) and of 34 (4.3–270; $P = 0.001$) in men (data not shown), which remained significant after correction for multiple comparisons; results in women were not significant (data not shown). In individuals with C282Y/C282Y, the adjusted odds ratio for hypertension using a cut-off of 140/90 mmHg was not significant versus wild type/wild type (Figure S1B); gender-stratified results were not significant (data not shown). Diastolic blood pressure, systolic blood pressure and pulse pressure as continuous traits did not differ between genotypes (data not shown). There was 80% power to detect an odds ratio of 4.6 for antihypertensive medication use and of 3.7 for hypertension using a cut-off of 140/90 in individuals with C282Y/C282Y genotype.

CGPS Of 36 480 individuals, 7080 ended the study receiving antihypertensive medication. The cumulative incidence of antihypertensive medication increased with C282Y/C282Y compared to wild type/-wild type (Figure S2A, Table 2). Adjusted hazard ratios for C282Y/C282Y versus wild type/wild type was 1.7 (1.2–2.3; $P = 0.003$) overall, and 2.1 (1.3–3.3; $P = 0.002$) in men (Table 2, Fig. 1a; both results were significant after correction for multiple comparisons); results in women were not significant. There was 80% power to detect a hazard ratio for risk of use of antihypertensive medication in individuals with C282Y/C282Y genotype of 1.7 overall and of 2.1 in men.

Absolute 10-year risk of treatment with antihypertensive medication for individuals with C282Y/C282Y was 4.3% for women and 5.6% for men (Fig. 2). Population-attributable risks for antihypertensive medication use for C282Y/C282Y genotype overall and in men were 0.2% and 0.4%, respectively.

Genotype and LVH

CCHS Amongst 8937 CCHS participants, 744 had ECG evidence of LVH. C282Y/C282Y was not associated with risk of LVH (Figure S1C). Adjusted odds ratio for LVH in C282Y/H63D versus wild type/wild type was 1.8 (1.1–3.1; $P = 0.02$) (Figure S1C) but was not significant after correction for multiple comparisons. There was 80% power to detect an odds ratio for LVH of 5.2 in C282/C282Y compared to wild type/-wild type.

Genotype and LVH with hypertension

CCHS Amongst 8937 CCHS participants, 402 had both LVH and hypertension. The odds ratio for LVH with hypertension in individuals with C282Y/C282Y versus wild type/wild type did not differ from 1.0 (data not shown).

LIFEGEN Diastolic blood pressure, systolic blood pressure, pulse pressure, Cornell voltage-duration product and Sokolow-Lyon voltage did not vary across haemochromatosis genotypes in patients with hypertension and LVH (Table 3). None of the haemochromatosis genotypes was associated with severity of LVH (i.e., above median versus below median) using either Cornell voltage product or Sokolow-Lyon voltage criteria (Figure S3), or both criteria combined (data not shown).

Iron overload and hypertension and/or LVH

CCHS Iron overload (transferrin saturation ≥50% vs. <50%) was not associated with hypertension or LVH separately (Figure S4) or combined (data not shown). Diastolic blood pressure, systolic blood pressure and pulse pressure as continuous traits were not affected by the presence or absence of iron overload (data not shown).

CGPS The cumulative incidence of antihypertensive medication use increased with extreme iron overload with transferrin saturation ≥80% compared to <50% overall (Figure S2B, Table 4) and in men (Fig. 1b). Adjusted hazard ratios for transferrin saturation ≥80% compared to <50% was 1.6 (1.0–2.6; $P = 0.05$) overall (Table 4) and 2.6 (1.6–4.2; $P < 0.0001$) in men (Fig. 1b); the result in men was still significant after correction for multiple comparisons.

Adjusted hazard ratios for transferrin saturation ≥80% + C282Y/C282Y genotype compared to transferrin saturation <50% + wild type/wild type genotype was 2.3 (1.3–4.2; $P = 0.005$) overall (Table 4) and 3.1 (1.6–5.7; $P < 0.0001$) in men (Fig. 1c); results were still significant after correction for multiple comparisons. Results in women were not significant. The cumulative incidence of antihypertensive medication use increased only after 40 years of age; after

Table 2 *Risk of antihypertensive medication use according to haemochromatosis genotype in the Copenhagen General Population Study*

Genotype	Participants *N*	Antihypertensive medication *N*	Incidence rate (95% CI), events/1000 person-years	Log-rank *P*	Hazard ratio (95% CI)	*P*	Power
All							
Wild type/wild type	24 064	4631	3.4 (3.3–3.5)	–	1.0	–	–
H63D/wild type	7578	1485	3.5 (3.3–3.6)	0.3	1.0 (1.0–1.1)	0.3	1.1
H63D/H63D	675	139	3.6 (3.1–4.3)	0.3	1.1 (0.9–1.3)	0.3	1.3
C282Y/wild type	3455	670	3.4 (3.2–3.7)	0.6	1.0 (0.9–1.1)	0.6	1.1
C282Y/H63D	588	121	3.7 (3.1–4.4)	0.07	1.2 (1.0–1.4)	0.07	1.3
C282Y/C282Y	120	34	5.0 (3.6–7.0)	0.003	1.7 (1.2–2.3)	0.003	1.7
Any C282Y genotype	4163	825	3.4 (3.3–3.7)	0.6	1.0 (1.0–1.1)	0.5	1.1
Women							
Wild type/wild type	12 895	2464	3.4 (2.3–3.5)	–	1.0	–	–
H63D/wild type	4074	811	3.5 (3.3–3.8)	0.6	1.0 (0.9–1.1)	0.6	1.1
H63D/H63D	351	73	3.6 (2.9–4.6)	0.6	1.1 (0.8–1.4)	0.6	1.4
C282Y/wild type	1832	338	3.2 (2.9–3.6)	0.3	0.9 (0.8–1.1)	0.3	1.2
C282Y/H63D	310	66	3.8 (3.0–4.9)	0.1	1.2 (0.9–1.5)	0.1	1.5
C282Y/C282Y	64	16	4.4 (2.7–7.1)	0.2	1.3 (0.8–2.2)	0.2	2.1
Any C282Y genotype	2206	420	3.4 (3.1–3.7)	0.8	1.0 (0.9–1.1)	0.8	1.2
Men							
Wild type/wild type	11 169	2167	3.4 (3.3–3.5)	–	1.0	–	–
H63D/wild type	3504	674	3.4 (3.1–3.7)	0.3	1.0 (1.0–1.1)	0.3	1.1
H63D/H63D	324	66	3.6 (2.8–4.6)	0.4	1.1 (0.9–1.4)	0.4	1.5
C282Y/wild type	1623	332	3.6 (3.2–4.0)	0.7	1.0 (0.9–1.2)	0.7	1.2
C282Y/H63D	278	55	3.6 (2.7–4.6)	0.3	1.2 (0.9–1.5)	0.3	1.5
C282Y/C282Y	56	18	5.8 (3.6–9.2)	0.002	2.1 (1.3–3.3)	0.002	2.1
Any C282Y genotype	1957	405	3.6 (3.3–4.0)	0.2	1.1 (1.0–1.2)	0.2	1.2

Analyses included data from 36 480 individuals (19 526 women and 16 954 men). Hazard ratios are adjusted for age and gender (all), or age alone (women, men). Power: 80% power to detect a hazard ratio (two-sided $P \leq 0.05$).

55 years this was most pronounced for those with C282Y/C282Y and transferrin saturation ≥80% either separately or combined (Fig. 1, Figure S2). There was 80% power to detect a hazard ratio of 2.3 and 3.0 overall and in men, respectively, for antihypertensive medication use in individuals with transferrin saturation ≥80% + C282Y/C282Y genotype compared to transferrin saturation <50% + wild type/wild type genotype.

Absolute 10-year risk of antihypertensive medication use for individuals with transferrin saturation ≥80% vs. <50% was 1.0% for women (based on only one woman) and 7.1% for men (Fig. 2). Values for individuals with transferrin saturation ≥80% + C282Y/C282Y were 2.9% for women and 7.7% for men (Fig. 2). Population-attributable risks for antihypertensive medication use for transferrin saturation ≥80% overall and in men were 0.1% and 0.4%, respectively; for transferrin saturation ≥80% + C282Y/C282Y genotype, the population-attributable risks were 0.1% overall and 0.3% in men.

Discussion

We examined the association between haemochromatosis genotype C282Y/C282Y or extreme elevation in transferrin saturation (signalling iron overload) and hypertension and/or LVH in two independent general population studies (a cross-sectional study

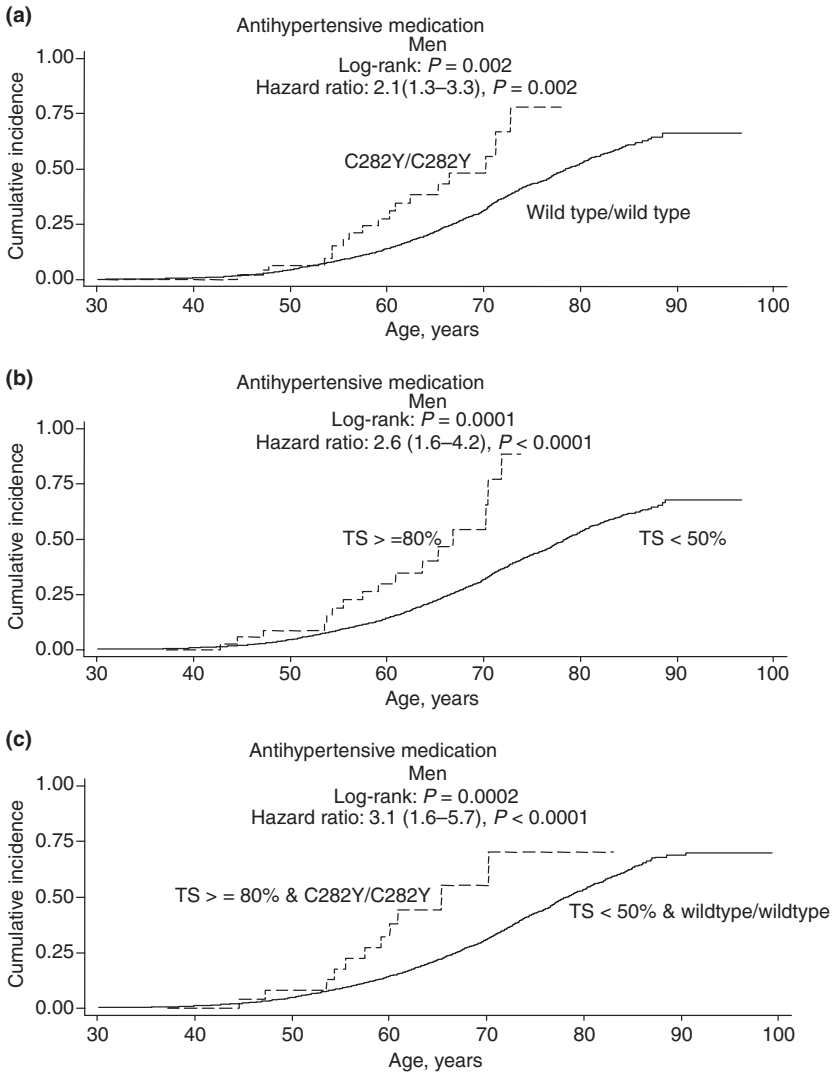

Fig. 1 *Cumulative incidence of antihypertensive medication use. C282Y haemochromatosis genotype (a), iron overload (b) or the combination of genotype and iron overload (c) in the Copenhagen General Population Study (CGPS) in men. TS, transferrin saturation.*

and a follow-up study) and in a case-only study of patients with hypertension and LVH. We found that haemochromatosis genotype C282Y/C282Y and extreme iron overload (transferrin saturation ≥80%) separately or combined were associated with increased risk of antihypertensive medication use overall and in men; although a lesser degree of iron overload was not associated with risk of antihypertensive treatment.

The significant finding in men but not in women is in accordance with previous findings that the genetic penetrance of haemochromatosis is higher in men than in women [34]. The fact that haemochromatosis is less penetrant in women than in men, does not, however explain why the association between transferrin saturation (>80%) and hypertension did not differ in women and men. Whether or not there is a protective effect of female gender, irrespective of iron overload, is not clear from our study. In addition, only a minority of women with transferrin saturation >80% were homozygous for C282Y suggesting that factors other than HFE haemochromatosis were able to induce iron overload in women (e.g. iron supplementation); however, we did not have the data to explore this further. Alternatively, as women with transferrin saturation of 70–80% showed a threefold risk of antihypertensive medication use, compared with those with a saturation of <50%, the lack of association with antihypertensive medication for the 17

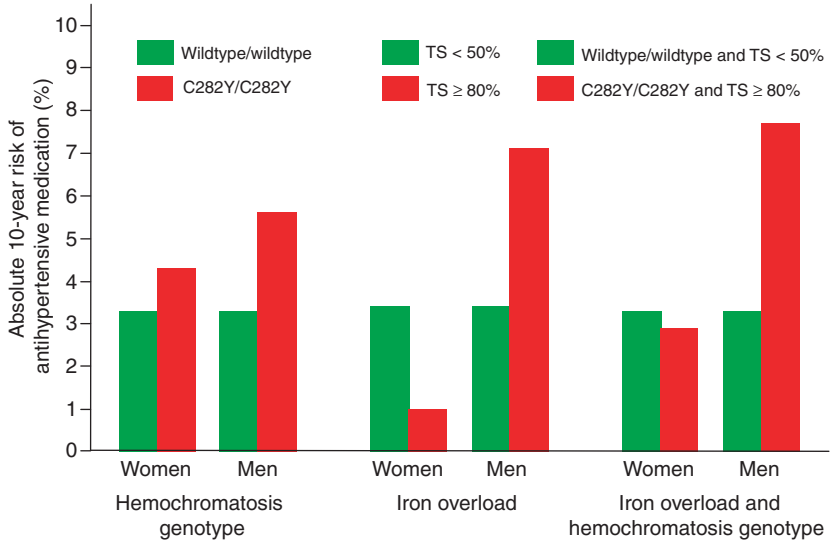

Fig. 2 *Absolute 10-year risk of antihypertensive medication use. Risk is stratified by haemochromatosis genotype, iron overload (TS, transferrin saturation) or the combination of genotype and iron overload according to gender.*

Table 3 *Blood pressure and ECG characteristics of patients in LIFEGEN according to haemochromatosis genotype*

	Wild type/ wild type *N* = 2576	H63D/ wild type *N* = 766	H63D/ H63D *N* = 74	C282Y/ wild type *N* = 338	C282Y/ H63D *N* = 52	C282Y/ C282Y *N* = 9	*P*-value
Diastolic blood pressure (mmHg)	100 (94–105)	100 (95–105)	100 (95–102)	100 (96–107)	100 (90–110)	103 (93–113)	0.6
Systolic blood pressure (mmHg)	178 (168–188)	175 (164–185)	183 (174–190)	178 (165–189)	173 (170–185)	176 (175–176)	0.5
Pulse pressure (mmHg)	78 (69–89)	77 (65–88)	84 (73–90)	75 (63–89)	78 (73–82)	73 (63–82)	0.4
Cornell voltage-duration product (mm × ms)	2648 (2274–3081)	2592 (2160–3036)	2711 (2280–3536)	2668 (2248–3116)	2688 (2173–3248)	3055 (2700–3410)	0.6
Sokolow-Lyon voltage (mm)	29 (22–37)	31 (23–38)	28 (22–30)	30 (23–38)	21 (19–32)	25 (22–27)	0.1

Values are medians (interquartile ranges). *P*-value: Kruskal–Wallis test across genotypes. For participants who were taking antihypertensive medication, we added 10 mmHg to the observed systolic blood pressure values and 5 mmHg to the observed diastolic blood pressure values [30, 31] in the analyses of blood pressure as continuous traits. A total of 2502 participants received antihypertensive treatment at baseline, i.e., before randomization.
LIFEGEN, Losartan Intervention For End-point Reduction in Hypertension Genetic Substudy.

women with a transferrin saturation >80% could also represent a chance finding. The sharp increase in antihypertensive medication use in those with C282Y/C282Y and transferrin saturation ≥80% either separately or combined mainly above 55 years of age is in accordance with previous findings that this is the most common age for the onset of symptomatic organ disease in hereditary haemochromatosis due to C282Y/C282Y [1]. Though the presence of a high transferrin saturation can be associated with a more expressed phenotype in haemochromatosis, it cannot give a true measure of iron overload alone; in this case ferritin levels would also be needed. Furthermore, high transferrin saturation need not be due to genetically defined haemochromatosis to be of pathogenetic importance.

It is indeed likely that haemochromatosis genotype C282Y/C282Y is associated with an increased risk of severe hypertension (i.e., use of antihypertensive medication), because there is the strongest evidence for this genotype of iron overload. Furthermore,

Table 4 *Risk of antihypertensive medication use according to levels of transferrin saturation in the Copenhagen General Population Study*

Transferrin saturation	Participants *N*	Antihypertensive medication *N*	Incidence rate (95% CI), events/1000 person-years	Log-rank p	Hazard ratio (95% CI)	*P*	Power
All							
<50%	35 946	6966	3.4 (3.3–3.5)	–	1.0	–	–
≥50% and <60%	328	67	3.6 (2.8–4.5)	0.8	1.0 (0.8–1.3)	0.7	1.4
≥60% and <70%	99	19	3.4 (2.2–5.3)	0.9	1.0 (0.7–1.6)	0.9	1.9
≥70% and <80%	35	8	4.2 (2.1–8.5)	0.3	1.4 (0.7–2.9)	0.3	2.7
≥80%	55	17	5.4 (3.3–8.7)	0.05	1.6 (1.0–2.6)	0.05	2.3
Trend, *P*						0.05	
Women							
<50%	19 350	3734	3.4 (3.3–3.5)	–	1.0	–	–
≥50% and <60%	97	20	3.7 (2.4–5.7)	0.5	1.1 (0.7–1.8)	0.5	1.9
≥60% and <70%	39	7	3.1 (1.5–6.5)	0.6	0.8 (0.4–1.8)	0.6	2.6
≥70% and <80%	13	5	7.8 (3.1–18)	0.005	3.3 (1.4–8.0)	0.007	3.6
≥80%	17	1	1.0 (0.1–7.3)	0.1	0.2 (0.1–1.6)	0.1	3.6
Trend, *P*						0.8	
Men							
<50%	16 596	3232	3.4 (3.3–3.5)	–	1.0	–	–
≥50% and <60%	231	47	3.5 (2.6–4.7)	0.9	1.0 (0.7–1.3)	0.9	1.5
≥60% and <70%	60	12	3.6 (2.0–6.3)	0.5	1.2 (0.7–2.1)	0.5	2.2
≥70% and <80%	22	3	2.4 (0.8–7.8)	0.6	0.7 (0.2–2.3)	0.6	3.4
≥80%	38	16	7.3 (4.5–12)	0.0001	2.6 (1.6–4.2)	<0.0001	2.6
Trend, *P*						0.008	
Only C282Y/C282Y[a]							
All							
<50% & wild type/wild type	23 898	4592	3.4 (3.3–3.5)	–	1.0	–	–
≥80% & C282Y/C282Y	28	11	6.9 (3.8–12)	0.005	2.3 (1.3–4.2)	0.005	2.3
Women							
<50% & wild type/wild type	12 841	2456	3.4 (3.3–3.5)	–	1.0	–	–
≥80% & C282Y/C282Y	6	1	2.9 (0.4–21)	0.7	0.7 (0.1–4.7)	0.7	6.0
Men							
<50% & wild type/wild type	11 057	2136	3.4 (3.2–3.5)	–	1.0	–	–
≥80% & C282Y/C282Y	22	10	8.0 (4.3–15)	0.0002	3.1 (1.6–5.7)	<0.0001	3.0

[a]Comparisons of C282Y/C282Y and transferrin saturation ≥80% versus wild type/wild type and transferrin saturation <50%. Analyses included data from 36 463 individuals (19 516 women and 16 947 men). Hazard ratios are adjusted for age and gender (all), or age alone (women, men). Power: 80% power to detect a hazard ratio (two-sided $P \leq 0.05$).

presence of the C282Y/C282Y genotype explains 83% of hereditary haemochromatosis [3], also supporting the concept that this genotype could associate with other clinical symptoms. That C282Y/wild type as well as the other haemochromatosis genotypes is not associated with hypertension also seems plausible because there is only modest evidence of iron overload for these genotypes, and generally they do not cause hereditary haemochromatosis [3]. However, some studies have shown a

higher prevalence of C282Y/wild type in disorders associated with mild iron overload; for example, porphyria cutanea tarda [7], Hepatitis C Virus-related hepatitis [35] and nonalcoholic steatohepatitis [36].

It could appear contradictory that C282Y/C282Y is associated with use of antihypertensive medication, but not with hypertension based on a single blood pressure measurement or with blood pressure on a continuous scale in the general population. However, use of antihypertensive medication probably indicates that a patient's general practitioner has found evidence of hypertension on several occasions, and therefore antihypertensive medication in the CCHS and CGPS is likely to be the best indicator of severe hypertension. Otherwise hypertension was recorded as blood pressure measurement on a single occasion in CCHS. Misclassification is likely from a single blood pressure measurement makes, as a result of a substantial variability in the measurement, a white coat effect and the possibility of masked hypertension. Thus, antihypertensive medication use may represent verified severe hypertension, whereas hypertension based on a single measurement may not.

The cause of the association between C282Y/C282Y genotype and treatment with antihypertensive medication may be extreme iron overload accumulated over a long period. Previous findings have shown an association between C282Y/C282Y [7] or iron overload [16, 37, 38] and features of the metabolic syndrome including hypertension. However, it is worth noting that increased transferrin saturation measured in this study (generally thought to represent an increased intestinal iron absorption typical of haemochromatosis) is different from hyperferritinaemia alone, which is a common presentation of the so-called dysmetabolic iron overload syndrome not related to haemochromatosis and in which transferrin saturation is often within the normal range [39]. A functional explanation of the detected association may be that long-term iron overload generates reactive oxygen species by the Fenton reaction, creating oxidative stress that increases vascular tone [14] and consequently results in hypertension.

As the susceptibility for hypertension (determined by the use of antihypertensive medication) observed in this study may be a genetic factor causing iron overload for which there is a treatment (phlebotomy), 0.1–0.4% of cases of hypertension could be resolved if patients received such treatment. Extrapolating these results to all patients with hypertension in Denmark, 200–600 patients per million persons could be helped with phlebotomy.

A limitation of the CCHS and CGPS was the lack of measurement of ferritin levels to estimate the extent of iron overload; however, elevated transferrin saturation, another marker of iron overload, is clearly associated with haemochromatosis genotypes. Likewise, a limitation of LIFEGEN was the lack of measurement of transferrin saturation as well as ferritin levels as C282Y homozygous genotype is not always associated with significant iron overload [34, 40]. Twenty-four-hour ambulatory measurement and home readings are better predictors of cardiovascular risk than a single blood pressure measurement; however, these standards were not part of the CCHS or CGPS protocol [41].

In conclusion, we found that haemochromatosis genotype C282Y/C282Y and extreme iron overload separately or combined are associated with increased risk of use of antihypertensive medication. Therefore, testing for haemochromatosis genotype C282Y/C282Y and extreme iron overload could be considered for patients with essential hypertension.

Funding

The Danish Heart Foundation and Chief Physician Johan Boserup's and Lise Boserup's Fund supported the CCHS. LIFEGEN was supported by an unrestricted grant from Merck.

Conflict of interest

None declared.

Acknowledgements

We thank Nina Dahl Kjersgaard, Jesper Schou and Mette Refstrup for technical assistance with genotyping assays.

References

1 Pietrangelo A. Hereditary hemochromatosis – a new look at an old disease. *N Engl J Med* 2004; **350:** 2383–97.
2 Beutler E, Bothwell TH, Charlton RW, Motulsky AG. Hereditary hemochromatosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease*. New York, NY: McGraw-Hill, 2001; 3127–62.
3 Feder JN, Gnirke A, Thomas W *et al.* A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996; **13:** 399–408.

4 Cecchetti G, Binda A, Piperno A, Nador F, Fargion S, Fiorelli G. Cardiac alterations in 36 consecutive patients with idiopathic haemochromatosis: polygraphic and echocardiographic evaluation. *Eur Heart J* 1991; **12:** 224–30.
5 Click RL, Olson LJ, Edwards WD *et al.* Echocardiography and systemic diseases. *J Am Soc Echocardiogr* 1994; **7:** 201–16.
6 Summary of the 2007 European Society of Hypertension (ESH) and European Society of Cardiology (ESC) guidelines for the management of arterial hypertension. *Vasc Health Risk Manag* 2007; **3:** 783–95.
7 Ellervik C, Birgens H, Tybjaerg-Hansen A, Nordestgaard BG. Hemochromatosis genotypes and risk of 31 disease endpoints: meta-analyses including 66,000 cases and 226,000 controls. *Hepatology* 2007; **46:** 1071–80.
8 van der AD, Rovers MM, Grobbee DE *et al.* Mutations in the HFE gene and cardiovascular disease risk. An individual patient data meta-analysis of 53 880 subjects. *Circ Cardiovasc Genet* 2008; **1:** 43–50.
9 Danesh J, Appleby P. Coronary heart disease and iron status: meta-analyses of prospective studies. *Circulation* 1999; **99:** 852–4.
10 Peterlin B, Globocnik PM, Makuc J, Hawlina M, Petrovic D. A hemochromatosis-causing mutation C282Y is a risk factor for proliferative diabetic retinopathy in Caucasians with type 2 diabetes. *J Hum Genet* 2003; **48:** 646–9.
11 Moczulski DK, Grzeszczak W, Gawlik B. Role of hemochromatosis C282Y and H63D mutations in HFE gene in development of type 2 diabetes and diabetic nephropathy. *Diabetes Care* 2001; **24:** 1187–91.
12 Oliva R, Novials A, Sanchez M *et al.* The HFE gene is associated to an earlier age of onset and to the presence of diabetic nephropathy in diabetes mellitus type 2. *Endocrine* 2004; **24:** 111–4.
13 Davis TM, Beilby J, Davis WA *et al.* Prevalence, characteristics, and prognostic significance of HFE gene mutations in type 2 diabetes: the Fremantle Diabetes Study. *Diabetes Care* 2008; **31:** 1795–801.
14 Cai H. Hydrogen peroxide regulation of endothelial function: origins, mechanisms, and consequences. *Cardiovasc Res* 2005; **68:** 26–36.
15 Valko M, Morris H, Cronin MT. Metals, toxicity and oxidative stress. *Curr Med Chem* 2005; **12:** 1161–208.
16 Piperno A, Trombini P, Gelosa M *et al.* Increased serum ferritin is common in men with essential hypertension. *J Hypertens* 2002; **20:** 1513–8.
17 Failla M, Giannattasio C, Piperno A *et al.* Radial artery wall alterations in genetic hemochromatosis before and after iron depletion therapy. *Hepatology* 2000; **32:** 569–73.
18 Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 2007; **298:** 299–308.
19 Schnohr P, Jensen JS, Scharling H, Nordestgaard BG. Coronary heart disease risk factors ranked by importance for the individual and community. A 21 year follow-up of 12 000 men and women from The Copenhagen City Heart Study. *Eur Heart J* 2002; **23:** 620–6.
20 Ellervik C, Mandrup-Poulsen T, Nordestgaard BG *et al.* Prevalence of hereditary haemochromatosis in late-onset type 1 diabetes mellitus: a retrospective study. *Lancet* 2001; **358:** 1405–9.
21 Andersen RV, Tybjaerg-Hansen A, Appleyard M, Birgens H, Nordestgaard BG. Hemochromatosis mutations in the general population: iron overload progression rate. *Blood* 2004; **103:** 2914–9.
22 Dahlof B, Devereux R, de Faire U *et al.* The Losartan Intervention For Endpoint reduction (LIFE) in Hypertension study: rationale, design, and methods. The LIFE Study Group. *Am J Hypertens* 1997; **10:** 705–13.
23 Dahlof B, Devereux RB, Julius S *et al.* Characteristics of 9194 patients with left ventricular hypertrophy: the LIFE study. Losartan Intervention For Endpoint Reduction in Hypertension. *Hypertension* 1998; **32:** 989–97.
24 Dahlof B, Devereux RB, Kjeldsen SE *et al.* Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet* 2002; **359:** 995–1003.
25 Appleyard M, Tybjærg Hansen A, Jensen G, Schnohr P, Nyboe J. The Copenhagen City Heart Study: Østerboundersøgelsen: a book of tables with data from the first examination (1976–78) and a five year follow up (1981–1983). *Scand J Soc Med Suppl* 1989; **41:** 1–160.
26 Schnohr P, Jensen G, Lange P, Scharling H, Appleyard M. The Copenhagen City Heart Study – tables with data from the third examination 1991–1994. *Eur Heart J Suppl* 2001; **3:** 1–83.
27 Sethi AA, Nordestgaard BG, Agerholm-Larsen B, Frandsen E, Jensen G, Tybjaerg-Hansen A. Angiotensinogen polymorphisms and elevated blood pressure in the general population: the Copenhagen City Heart Study. *Hypertension* 2001; **37:** 875–81.
28 Blackburn H, Keys A, Simonson E, Rautaharju P, Punsar S. The electrocardiogram in population studies. A classification system. *Circulation* 1960; **21:** 1160–75.
29 Baty D, Terron KA, Mechan D, Harris A, Pippard MJ, Goudie D. Development of a multiplex ARMS test for mutations in the HFE gene associated with hereditary haemochromatosis. *J Clin Pathol* 1998; **51:** 73–4.
30 Cui JS, Hopper JL, Harrap SB. Antihypertensive treatments obscure familial contributions to blood pressure variation. *Hypertension* 2003; **41:** 207–10.
31 Levy D, Ehret GB, Rice K *et al.* Genome-wide association study of blood pressure and hypertension. *Nat Genet* 2009; **41:** 677–87.
32 Harrison-Findik DD. Role of alcohol in the regulation of iron metabolism. *World J Gastroenterol* 2007; **13:** 4925–30.
33 Wall JD, Pritchard JK. Haplotype blocks and linkage disequilibrium in the human genome. *Nat Rev Genet* 2003; **4:** 587–97.
34 Allen KJ, Gurrin LC, Constantine CC *et al.* Iron-overload-related disease in HFE hereditary hemochromatosis. *N Engl J Med* 2008; **358:** 221–30.
35 Smith BC, Gorve J, Guzail MA *et al.* Heterozygosity for hereditary hemochromatosis is associated with more fibrosis in chronic hepatitis C. *Hepatology* 1998; **27:** 1695–9.
36 George DK, Goldwurm S, MacDonald GA *et al.* Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. *Gastroenterology* 1998; **114:** 311–8.
37 Vari IS, Balkau B, Kettaneh A *et al.* Ferritin and transferrin are associated with metabolic syndrome abnormalities and their change over time in a general population: Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR). *Diabetes Care* 2007; **30:** 1795–801.
38 Jehn M, Clark JM, Guallar E. Serum ferritin and risk of the metabolic syndrome in U.S. adults. *Diabetes Care* 2004; **27:** 2422–8.
39 Moirand R, Mortaji AM, Loreal O, Paillard F, Brissot P, Deugnier Y. A new syndrome of liver iron overload with normal transferrin saturation. *Lancet* 1997; **349:** 95–7.
40 Deugnier Y, Brissot P, Loreal O. Iron and the liver: update 2008. *J Hepatol* 2008; **48 Suppl. 1:** S113–23.

41 Pickering TG, Hall JE, Appel LJ *et al.* Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Hypertension* 2005; **45:** 142–61.

Correspondence: Børge G. Nordestgaard, MD, DMSc, Professor, Chief Physician, Department of Clinical Biochemistry, Herlev Hospital, Herlev Ringvej 75, DK-2730 Herlev, Denmark.
(fax: +45 44883311; e-mail: brno@heh.regionh.dk).

Supporting Information

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

Figure S1. Risk of antihypertensive medication use (A), hypertension (B) and left ventricular hypertrophy (C). Risk by haemochromatosis genotype in the Copenhagen City Heart Study (CCHS). The power indicates the odds ratio that can be detected at 80% (two-sided $P \leq 0.05$).

Figure S2. Cumulative incidence of antihypertensive medication use. C282Y haemochromatosis genotype (A) or iron overload (B) in the Copenhagen General Population Study (CGPS). TS, transferrin saturation.

Figure S3. Risk of severe left ventricular hypertrophy by haemochromatosis genotype in LIFEGEN. LIFEGEN, Losartan Intervention For End-point Reduction in Hypertension Genetic Substudy. The power indicates the odds ratio that can be detected at 80% (two-sided $P \leq 0.05$).

Figure S4. Risk of antihypertensive medication use, hypertension and left ventricular hypertrophy (LVH). Risk stratified by iron overload [transferrin saturation (TS) ≥50% vs. <50%] in the Copenhagen City Heart Study (CCHS). The power indicates the odds ratio that can be detected at 80% (two-sided $P \leq 0.05$).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.