gene mutations increase the risk of coronary heart disease in women

M. Carolina Pardo Silva, Omer T. Njajou, Behrooz Z. Alizadeh, Albert Hofman, Jacqueline C. M. Witteman, Cornelia M. Duijn, A. Cecile J. W. Janssens

HFE Gene Mutations Increase the Risk of Coronary Heart Disease in Women

Running head: *HFE* mutations and CHD risk

M. Carolina Pardo Silva MD, MSc[*]

Omer T. Njajou PhD[*]

Behrooz Z. Alizadeh MD, PhD[*]

Albert Hofman MD, PhD[*]

Jacqueline C.M. Witteman PhD[*]

Cornelia M. van Duijn PhD[*]

A. Cecile J.W. Janssens PhD[*]

*From the Department of Epidemiology, Erasmus University Medical Center Rotterdam, the Netherlands.

Correspondence to:

Dr A.Cecile J. W. Janssens

Department of Epidemiology,

Erasmus University Medical Center Rotterdam

P.O. Box 2040, 3000 CA, Rotterdam, the Netherlands.

Phone number +31 (10) 7044214

Fax Number +31 (10) 7044657

Email: a.janssens@erasmusmc.nl

Abstract

The purpose of the present study is to examine *HFE* gene mutations in relation to newly diagnosed (incident) coronary heart disease (CHD). In a population-based follow-up study of 7,983 individuals aged 55 years and older, we compared the risk of incident CHD between *HFE* carriers and non-carriers, overall and stratified by sex and smoking status. *HFE* mutations were significantly associated with an increased risk of incident CHD in women but not in men (hazard ratio [HR] for women = 1.7, 95% confidence interval [CI] 1.2 to 2.4 versus HR for men = 0.9, 95% CI 0.7 to 1.2). This increased CHD risk associated with *HFE* mutations in women was statistically significant in never smokers (HR = 1.8, 95% CI 1.1 to 2.8) and current smokers (HR = 3.1, 95% CI 1.4 to 7.1), but not in former smokers (HR = 1.3, 95% CI 0.7 to 2.4). *HFE* mutations are associated with increased risk of incident CHD in women.

Introduction

Hereditary hemochromatosis is a genetic disorder characterized by iron overload [1]. Iron overload in patients with hereditary hemochromatosis results in iron depots in the pancreas, liver, joints and heart. In the heart, such depots have been associated with coronary heart disease (CHD) and shortened life expectancy [2]. The most common cause of hereditary hemochromatosis are two common mutations in the *HFE* gene, C282Y and H63D [3,4]. Over 80% of the patients with hereditary hemochromatosis are homozygous for the C282Y mutation, about 1% are homozygous for the H63D mutation and about 7% are compound heterozygous [1]. In populations of European origin, an estimated 0.4% of the population is homozygous and 9% is heterozygous carrier of the C282Y mutation and 13% is homozygous and 2% heterozygous for the H63D mutation [1].

Complications of iron overload may not only occur to homozygous carriers of the risk alleles. Compound heterozygotes and heterozygotes for the C282Y and H63D mutations also have a subtle increases in serum iron, serum ferritin and transferrin saturation [5,6,7,8]. These slight changes suggest that heterozygotes are more likely to have a slow accumulation of iron, which may lead to pathology later in life**. Iron deposits in the arterial wall trigger the low density lipoprotein cholesterol peroxidation and therefore contributes the formation of atherosclerotic lesions and to the inflammation leading to cardiovascular disease.[1] [2] Some studies also showed that C282Y carriers had an increased risk of myocardial infarction,[3] and coronary heart disease (CHD).[4] Similarly, smoking has also been associated to cardiovascular disease through increased inflammation, thrombosis, and oxidation of low-density lipoprotein cholesterol. [6] Therefore, to properly disentangle the association between *HFE* mutations and cardiovascular disease requires taking into account the effect of smoking. Following this, previous work of our group also showed an increased risk of stroke among *HFE* carriers who smoked.[5]** The aim of the present

study was to examine the effect of the two common *HFE* C282Y and H63D mutations on incident CHD **accounting for the effect of smoking.** Since women are protected early in life from iron-related pathology by menstruation, we examined men and women separately.

Methods

Study population

The present analysis was performed within the Rotterdam Study, an ongoing population-based study on the determinants of disease and disability in 7,983 subjects aged 55 years and older. Design, rationale and details of the study have been described previously [24]. The present analyses used baseline data (collected between 1990 and 1993) and follow-up morbidity and mortality data (collected until December 2001; mean follow-up of 8.3 years, standard deviation (SD) 2.7 years). The Medical Ethics Committee of the Erasmus MC University Medical Center approved the study protocol and all participants provided written informed consent.

Data collection

Baseline data collection

During home visits, a trained interviewer obtained information on health status, medical history, medication use and smoking status at baseline. Subsequently, participants were invited to the study center where they underwent an extensive clinical examination in which height, weight, systolic and diastolic blood pressures were measured. Body mass index (BMI) was computed as weight (kg) divided by height squared (m^2). Systolic and diastolic blood pressures were measured twice in sitting position, after 5 minutes rest, using a random-zero sphygmomanometer. The mean of the two measurements was used for the analysis. Hypertension was defined as systolic blood pressure higher than 160 mm Hg, diastolic blood pressure higher than 100 mm Hg or the use of medication indicated to treat high blood pressure (hypertension grades 2 and 3) [25]. Serum glucose, total cholesterol, high-density lipoproteins (HDL) cholesterol and C-reactive protein levels, as a marker of inflammation, were determined using an automated enzymatic procedure [26,27]. Diabetes was diagnosed

based on the use of medication, and/or a random or post-load glucose levels higher than 11.1 mmol/L [28]. Iron and ferritin levels and transferrin saturation were determined in subgroup as previously described [6].

Follow-up assessment

During follow-up, information on fatal and non-fatal cardiovascular endpoints was obtained from the general practitioners and hospital records. Two research physicians and a cardiologist independently reviewed all information and classified all the events according to the International Classification of Diseases, 10th edition (ICD-10) [29]. Incident CHD was defined as the occurrence of non-fatal myocardial infarction (ICD-10 code I21), revascularization procedure (percutaneous transluminal coronary angioplasty or coronary artery bypass graft) and cardiac death. Cardiac death was defined as death caused by myocardial infarction or other ischemic heart disease (ICD-10 codes I20 - I25), sudden cardiac death (ICD-10 code I46), cardiac arrhythmias (ICD-10 code I49), sudden death undefined (ICD-10 code R96) or death from heart failure (ICD-10 code I50).

Genotyping of HFE mutations

C282Y and H63D mutations were genotyped in a random sample of 3,798 individuals as described in details previously [22]. Baseline characteristics did not differ between randomly selected and non-selected participants.

Statistical analysis

From the 3,798 individuals who had C282Y and H63D genotype available, 3,435 had complete information on cardiovascular risk factors at baseline and were included in the analyses. There were only 10 homozygotes for the C282Y and 80 homozygotes for the H63D

mutation among which 2 and 11 cases of incident CHD respectively. Previous studies on the *HFE* mutations and iron parameters showed that serum iron, serum ferritin and transferrin saturation were similar for C282Y and H63D heterozygotes [5,6]. Therefore, we pooled carriers of C282Y or H63D mutations as *HFE* carriers. Differences in baseline characteristics between the *HFE* carriers and non-carriers were tested using chi-squared statistics (for categorical variables) or t-tests (for continuous variables). Variables that were not normally distributed, are presented as median with interquartile range and analyzed using the Mann-Whitney-U test. CHD incidence rates were calculated as number of events per 1,000 person-years. Risks of incident CHD were quantified as hazard ratios (HRs) from Cox proportional hazards models using age as time scale [30]. Survival time was calculated from age at baseline to age at event. HRs were calculated crude and adjusted for sex, smoking status, hypertension, BMI, total serum cholesterol, HDL cholesterol, diabetes mellitus and CHD at baseline. Further, HRs were additionally adjusted for C reactive protein, to evaluate the role of inflammation in the relationship between *HFE* mutations and incident CHD. The association between *HFE* mutations and the risk of incident CHD was investigated overall and in sex and smoking subgroups given that both are associated with CHD and the clinical manifestations of iron overload in our study [22]. Proportionality of all models was tested by Schoenfeld residuals [31]. P-values lower then 0.05 were considered statistically significant.

Results

Genotypes and allele frequencies were in Hardy-Weinberg equilibrium (C282Y: CC 88.1%, CY 11.6%, YY 0.3%, p = 0.41; H63D: HH 73.2%, HD 24.5%, DD 2.4%, p = 0.25). At baseline, women had lower prevalence of smoking, hypertension and myocardial infarction and a higher mean BMI compared to men. Baseline characteristics by genotype are presented in Table 1. Carriers and non-carriers of the *HFE* mutations did not significantly differ in risk

factors for cardiovascular disease with three exceptions in women. In women, *HFE* carriers had higher median body mass index (27.0 versus 26.6 kg/m^2; p=0.05), higher median C-reactive protein levels than non-carriers (2.0 versus 1.7 mg/dL; p = 0.05) and a lower frequency of diabetes mellitus (10% versus 13%; p=0.05). *HFE* carriers had higher levels of iron and ferritin and higher transferrin saturation than non-carriers (Table 2). This difference was statistically significant for both iron levels and transferrin saturation in men and women. A similar trend was observed within smoking strata, although the differences were not statistically significant (Data not shown). Figure 1 shows that females *HFE* carriers tend to have higher C-reactive protein levels than non-carriers, particularly among current or former smokers (p=0.08). In men, no differences in C-reactive protein levels between *HFE* carriers and non-carriers were observed.

During follow-up, 483 participants developed incident CHD. Men had higher CHD incidence than women (25.3 versus 11.8 cases/1,000 person years, p <0.001; Table 3). *HFE* mutations were associated with an increased risk of incident CHD in women **(HR_{crude} [95% CI] = 1.3 [1.0 to 1.8]), but not in men (HR_{crude} [95% CI] = 0.9 [0.7 to 1.2]). Further adjustment by co-variates including smoking status lead to an increase in the risk of CHD in women ($HR_{adjusted}$ [95% CI] = 1.7 [1.2 to 2.4]), but not in men ($HR_{adjusted}$ [95% CI] = 0.9 [0.7 to 1.2]). Additionally to HFE status, history of myocardial infarction, diabetes mellitus at baseline and smoking were important risk factors for CHD in women. In men, serum cholesterol level and high-density lipoprotein level, diabetes mellitus and history of myocardial infarction were key risk factors for CHD.** This increased CHD risk associated with *HFE* mutations in women was statistically significant in current smokers (HR = 3.1, 95% CI 1.4 to 7.1) and never smokers (HR = 1.8, 95% CI 1.1 to 2.8), but not in former smokers (HR = 1.3, 95% CI 0.7 to 2.4; Table 4). Exclusion of the homozygous and compound heterozygous carriers did not change the results (Data not

shown). **The interaction term HFE status * smoking status was not statistically significant.**

Discussion

We have found that the *HFE* mutations are associated with higher risk of incident CHD in women. C-reactive protein, a strong predictor of CHD risk was not associated with *HFE* mutations in women with current or former smoking history. Our study was embedded in a large follow-up study of Caucasian individuals aged 55 and older. This long follow-up allows us to study outcomes that are the result of lifelong exposures and express at older ages like cardiovascular disease associated with iron overload.

A previous report based on a smaller sample of the Rotterdam study (n = 342) showed that *HFE* genotypes, also those who were heterozygous, were associated with higher levels of transferrin saturation and serum iron levels, albeit that levels in those homozygous for the mutations are exponentially higher [6]. Previous work of our group also showed an increased risk of stroke among *HFE* carriers who smoked [22]. Altogether, these results are in line with the mechanism linking *HFE* mutations and smoking to cardio- and cerebrovascular disease through damage to vessel wall and inflammation [22]. The underlying mechanism linking *HFE* and CHD is thought to be through iron-related oxidative stress and the subsequent damage to vessel wall and inflammation [22,32]. Such mechanism is also supported by endothelial dysfunction observed in normal subjects after receiving intravenous iron sucrose reported recently [33], as well as the positive association between markers of oxidative stress and iron therapy [34]. Despite the solid grounds for this association, our data showed that CRP levels were not an intermediate factor in this association. A previous study on the association of chronic inflammation and hemochromatosis phenotype revealed that CRP levels did not change between homozygotes for the C282Y mutations with high or low iron stores [35]. Most likely, blood CRP level is a biomarker for generalized inflammation not specific to the association *HFE* and CHD. How and through which early intermediate inflammatory pathways *HFE* is related to inflammation, remains to be determined.

Another issue to be explained is the fact we only found significant evidence for association of *HFE* and CHD in women and not in men. While there is an ongoing discussion of the validity of subgroup testing by sex in genetic studies [36], there are reasons to separate men and women for *HFE*. First, phlebotomy is the key strategy for prevention of hemochromatosis and women are therefore naturally protected due to their menstruation [37]. While this is a common explanation for sex-differences in CHD risk associated with *HFE* mutations, in our study an increased risk was found in female carriers rather than in male carriers. This may be related to the study design. We studied elderly people and therefore male carriers may have been selected out from the population due to early mortality related to *HFE*. In women, the gene effect is likely to express clinically later as they are protected by menstruation up to menopause. **Second, it also should be noted that the major risks factors for CHD are smoking, body mass index, diabetes and lipid profile as supported by the effect of these co-variates when adjusting HR for CHD in women and men.** The extent of CHD risk conferred by these factors is of such magnitude that the genetic predisposition associated with *HFE* carrier status is rapidly outweighed as seen in Table 4 where it is shown that greater CHD risk is associated with smoking status rather than with *HFE* mutations. Given the higher prevalence of smoking (current and former) among men compared to women at baseline, it is likely that the modest effect of *HFE* mutations on the risk of CHD is only apparent among women.

Because of their role in iron overload and their impact on CHD risk, *HFE* mutations have been of interest in public health [7,38,39,40,41,42,43], For long, they have been viewed as a model for genetic testing. A genetic-based strategy for prevention of iron overload and its complications has been investigated after the identification of the two common mutations in the *HFE* gene [44]. The small effect size, the lack of consistency in the association between *HFE* mutations and cardiovascular disease and mortality [11,19,20,21], and the

possible effect modification by environmental factors such as smoking [11,17,19,20,21,22] implies that the benefit of such screening program varies between different subgroups, imposing further difficulties to the use of genetic testing for population-based screening. The results of our study confirm these previous findings and do not support genetic screening on *HFE* mutations.

Acknowledgements

The Rotterdam Study is supported by the Erasmus Medical Center and Erasmus University Rotterdam, the Netherlands Organization for Scientific Research (NWO), the Netherlands Organization for Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DGXII), the Municipality of Rotterdam, and the Centre for Medical Systems Biology (CMSB). This work was supported by an Erasmus University Rotterdam grant to Dr. Omer T. Njajou and by a ZonMW grant to Dr. Behrooz Z. Alizadeh. The contributions of the general practitioners and pharmacists of the Ommoord district to the Rotterdam Study are greatly acknowledged. We are grateful to JM Vergeer, JR Oskamp and WM Luijten for their help in the genotyping.

References

1. Hanson EH, Imperatore G, Burke W (2001) HFE gene and hereditary hemochromatosis: a HuGE review. Human Genome Epidemiology. Am J Epidemiol 154: 193-206.
2. Pietrangelo A (2004) Hereditary hemochromatosis--a new look at an old disease. N Engl J Med 350: 2383-2397.
3. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, et al. (1996) A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 13: 399-408.
4. Kotze MJ, de Villiers JN, Bouwens CS, Warnich L, Zaahl MG, et al. (2004) Molecular diagnosis of hereditary hemochromatosis: application of a newly-developed reverse-hybridization assay in the South African population. Clin Genet 65: 317-321.
5. Whitfield JB, Cullen LM, Jazwinska EC, Powell LW, Heath AC, et al. (2000) Effects of HFE C282Y and H63D polymorphisms and polygenic background on iron stores in a large community sample of twins. Am J Hum Genet 66: 1246-1258.
6. Njajou OT, Houwing-Duistermaat JJ, Osborne RH, Vaessen N, Vergeer J, et al. (2003) A population-based study of the effect of the HFE C282Y and H63D mutations on iron metabolism. Eur J Hum Genet 11: 225-231.
7. Burt MJ, George PM, Upton JD, Collett JA, Frampton CM, et al. (1998) The significance of haemochromatosis gene mutations in the general population: implications for screening. Gut 43: 830-836.
8. Gurrin LC, Bertalli NA, Dalton GW, Osborne NJ, Constantine CC, et al. (2009) HFE C282Y/H63D compound heterozygotes are at low risk of hemochromatosis-related morbidity. Hepatology 50: 94-101.
9. Bathum L, Christiansen L, Nybo H, Ranberg KA, Gaist D, et al. (2001) Association of mutations in the hemochromatosis gene with shorter life expectancy. Arch Intern Med 161: 2441-2444.
10. Carru C, Pes GM, Deiana L, Baggio G, Franceschi C, et al. (2003) Association between the HFE mutations and longevity: a study in Sardinian population. Mech Ageing Dev 124: 529-532.
11. Coppin H, Bensaid M, Fruchon S, Borot N, Blanche H, et al. (2003) Longevity and carrying the C282Y mutation for haemochromatosis on the HFE gene: case control study of 492 French centenarians. BMJ 327: 132-133.

12. Sullivan JL (1999) Iron and the genetics of cardiovascular disease. Circulation 100: 1260-1263.
13. de Valk B, Marx JJ (1999) Iron, atherosclerosis, and ischemic heart disease. Arch Intern Med 159: 1542-1548.
14. Tuomainen TP, Kontula K, Nyyssonen K, Lakka TA, Helio T, et al. (1999) Increased risk of acute myocardial infarction in carriers of the hemochromatosis gene Cys282Tyr mutation : a prospective cohort study in men in eastern Finland. Circulation 100: 1274-1279.
15. Gunn IR, Maxwell FK, Gaffney D, McMahon AD, Packard CJ (2004) Haemochromatosis gene mutations and risk of coronary heart disease: a west of Scotland coronary prevention study (WOSCOPS) substudy. Heart 90: 304-306.
16. Campbell S, George DK, Robb SD, Spooner R, McDonagh TA, et al. (2003) The prevalence of haemochromatosis gene mutations in the West of Scotland and their relation to ischaemic heart disease. Heart 89: 1023-1026.
17. Roest M, van der Schouw YT, de Valk B, Marx JJ, Tempelman MJ, et al. (1999) Heterozygosity for a hereditary hemochromatosis gene is associated with cardiovascular death in women. Circulation 100: 1268-1273.
18. Surber R, Sigusch HH, Kuehnert H, Figulla HR (2003) Haemochromatosis (HFE) gene C282Y mutation and the risk of coronary artery disease and myocardial infarction: a study in 1279 patients undergoing coronary angiography. J Med Genet 40: e58.
19. Beutler E, Felitti VJ (2002) The C282Y mutation does not shorten life span. Arch Intern Med 162: 1196-1197.
20. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T (2002) Penetrance of 845G--> A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet 359: 211-218.
21. Van Aken MO, De Craen AJ, Gussekloo J, Moghaddam PH, Vandenbroucke JP, et al. (2002) No increase in mortality and morbidity among carriers of the C282Y mutation of the hereditary haemochromatosis gene in the oldest old: the Leiden 85-plus study. Eur J Clin Invest 32: 750-754.
22. Njajou OT, Hollander M, Koudstaal PJ, Hofman A, Witteman JC, et al. (2002) Mutations in the hemochromatosis gene (HFE) and stroke. Stroke 33: 2363-2366.
23. Rasmussen ML, Folsom AR, Catellier DJ, Tsai MY, Garg U, et al. (2001) A prospective study of coronary heart disease and the hemochromatosis gene (HFE) C282Y

mutation: the Atherosclerosis Risk in Communities (ARIC) study. Atherosclerosis 154: 739-746.

24. Hofman A, Breteler MM, van Duijn CM, Krestin GP, Pols HA, et al. (2007) The Rotterdam Study: objectives and design update. Eur J Epidemiol 22: 819-829.
25. (1999) 1999 World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. Guidelines Subcommittee. J Hypertens 17: 151-183.
26. van Gent CM, van der Voort HA, de Bruyn AM, Klein F (1977) Cholesterol determinations. A comparative study of methods with special reference to enzymatic procedures. Clin Chim Acta 75: 243-251.
27. (2001) Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 285: 2486-2497.
28. (2003) Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 26 Suppl 1: S5-20.
29. WHO (1992) International Statistical classification of diseases and related health problems. Geneva: World Health Organization.
30. Korn EL, Graubard BI, Midthune D (1997) Time-to-event analysis of longitudinal follow-up of a survey: choice of the time-scale. Am J Epidemiol 145: 72-80.
31. Schoenfeld D (1982) Partial residuals for the proportional hazards regression model. Biometrika 69: 239-241.
32. Zacharski LR, Chow BK, Howes PS, Shamayeva G, Baron JA, et al. (2007) Reduction of iron stores and cardiovascular outcomes in patients with peripheral arterial disease: a randomized controlled trial. Jama 297: 603-610.
33. Zheng H, Huang X, Zhang Q, Katz SD (2006) Iron sucrose augments homocysteine-induced endothelial dysfunction in normal subjects. Kidney Int 69: 679-684.
34. Bayes B, Cruz Pastor M, Bonal J, Foraster A, Romero R (2005) Oxidative stress, inflammation and cardiovascular mortality in haemodialysis--role of seniority and intravenous ferrotherapy: analysis at 4 years of follow-up. Nephrol Dial Transplant.
35. Beutler E, Waalen J, Gelbart T (2005) Chronic inflammation does not appear to modify the homozygous hereditary hemochromatosis phenotype. Blood Cells Mol Dis 35: 326-327.
36. Patsopoulos NA, Tatsioni A, Ioannidis JP (2007) Claims of sex differences: an empirical assessment in genetic associations. Jama 298: 880-893.

37. Allen KJ, Gurrin LC, Constantine CC, Osborne NJ, Delatycki MB, et al. (2008) Iron-overload-related disease in HFE hereditary hemochromatosis. N Engl J Med 358: 221-230.

38. Burke W, Cogswell ME, MacDonnell SM, Franks AL (2000) Public health strategies to prevent complications of hemochromatosis. In: Khoury MJB, W. Thomson, E. eds, editor. Genetics and Public Health in the 21st Century. New York: Oxford University Press.

39. Burke W, Imperatore G, McDonnell SM, Baron RC, Khoury MJ (2000) Contribution of different HFE genotypes to iron overload disease: a pooled analysis. Genet Med 2: 271-277.

40. Cogswell ME, Burke W, McDonnell SM, Franks AL (1999) Screening for hemochromatosis. A public health perspective. Am J Prev Med 16: 134-140.

41. Khoury MJ, Burke W, Thomson E (2000) Genetics and public health: a framework for integration of human genetics into public health practices. In: Khoury MJB, W. Thomson, E. eds, editor. Genetics and public health in the 21st century. New York: Oxford University Press. pp. 3-24.

42. Khoury MJ, McCabe LL, McCabe ER (2003) Population screening in the age of genomic medicine. N Engl J Med 348: 50-58.

43. Whitlock EP, Garlitz BA, Harris EL, Beil TL, Smith PR (2006) Screening for hereditary hemochromatosis: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med 145: 209-223.

44. Burke W, Thomson E, Khoury MJ, McDonnell SM, Press N, et al. (1998) Hereditary hemochromatosis: gene discovery and its implications for population-based screening. JAMA 280: 172-178.

Table 1. **Baseline characteristics by sex and *HFE* carrier status**

		Women					Men				
		HFE-carriers (n=656)		Non-carriers (n=1,174)		p-Value	*HFE*-carriers (n=617)		Non-carriers (n=988)		p-Value
Age at entry (years)		69.3	(9.0)	69.6	(9.2)	0.46	67.7	(7.8)	68.0	(7.6)	0.39
Smoking status	Current smokers	105	(16%)	242	(21%)		193	(31%)	288	(29%)	
	Former smokers	187	(29%)	333	(28%)	0.43	367	(59%)	638	(65%)	0.46
	Never smokers	364	(55%)	599	(51%)		57	(9%)	62	(6%)	
Systolic blood pressure (mm Hg)		139.9	(21.7)	139.3	(23.2)	0.62	138.4	(21.3)	138.5	(22.5)	0.95
Diastolic blood pressure (mm Hg)		73.6	(11.5)	73.1	(11.6)	0.32	75.1	(11.8)	74.1	(11.7)	0.12
Hypertension		405	(62%)	735	(63%)	0.61	416	(68%)	675	(68%)	0.73
Body mass index (kg/m^2)		27.0	(4.3)	26.6	(3.9)	0.05	25.8	(3.0)	25.7	(3.0)	0.60
Diabetes mellitus		66	(10%)	154	(13%)	0.05	80	(13%)	111	(11%)	0.30
Total serum cholesterol (mmol/L)		6.83	(1.2)	6.85	(1.2)	0.72	6.28	(1.3)	6.35	(1.1)	0.26
HDL cholesterol (mmol/L)		1.44	(0.4)	1.42	(0.4)	0.41	1.19	(0.3)	1.22	(0.3)	0.07
C-reactive protein (mg/L)†		2.0	(0.9-3.7)	1.7	(0.9-3.3)	0.05	1.9	(0.9-3.9)	1.9	(0.9-3.8)	0.72
Myocardial infarction at baseline		66	(11%)	152	(13%)	0.18	147	(25%)	232	(24%)	0.74

Values are means (standard deviations) for continuous variables and numbers (percentages) for categorical variables. P-Values were obtained by t-test for continuous variables, $\chi 2$ for categorical variables, and Mann-Whitney U test for C-reactive protein. † Median (interquartile range).
HDL = High density lipoprotein

Table 2. **Iron parameters by sex and *HFE* carrier status**

	Women			Men		
	HFE-carriers (n=37)	Non-carriers (n=130)	p-Value	*HFE*-carriers (n=35)	Non-carriers (n=124)	p-Value
Iron (umol/L)	18.0 (14.0 – 21.0)	14.0 (13.0 - 18.5)	0.02	20.0 (15.3 – 23.0)	16.0 (13.0 – 20.0)	<0.01
Ferritin (ug/L)	141.5 (79.5 - 218.0)	109.0 (76.5 - 223.0)	0.39	194.0 (107.0 - 358.0)	187.0 (86.0 - 275.0)	0.22
Transferrin saturation (%)	29.9 (23.6 - 35.5)	23.4 (20.1 - 29.1)	<0.01	33.7 (25.4 - 41.8)	27.2 (22.0 - 31.6)	<0.01

Values are medians (interquartile range). P-Values were obtained by Mann-Whitney U test.

Table 3. **Incidence rates and hazard ratios for incident coronary heart disease by sex and *HFE* carrier status**

		CHD	Follow-up (py)	Incidence rate (CHD/1,000 py)	Hazard ratio (95% CI) Crude	Adjusted*
Women	All	177	15,020	11.8 (10.2 to 13.7)		
	HFE-carriers	73	5,370	13.6 (**10.8** to 17.1)	1.3 (1.0 to 1.8)	1.7 (1.2 to 2.4)
	Non-carriers	104	9,650	10.8 (**8.9** to 13.1)		
Men	All	306	12,093	25.3 (22.6 to 28.3)		
	HFE-carriers	116	4,735	24.5 (**20.4** to 29.4)	0.9 (0.7 to 1.2)	0.9 (0.7 to 1.2)
	Non-carriers	190	7,358	25.8 (**22.4** to 29.8)		

*** Adjusted for body mass index, hypertension, diabetes mellitus, smoking status at baseline, total and high density lipoprotein cholesterol, C - reactive protein and myocardial infarction at baseline. CHD: Coronary heart disease, py: person-years, CI: confidence interval.**

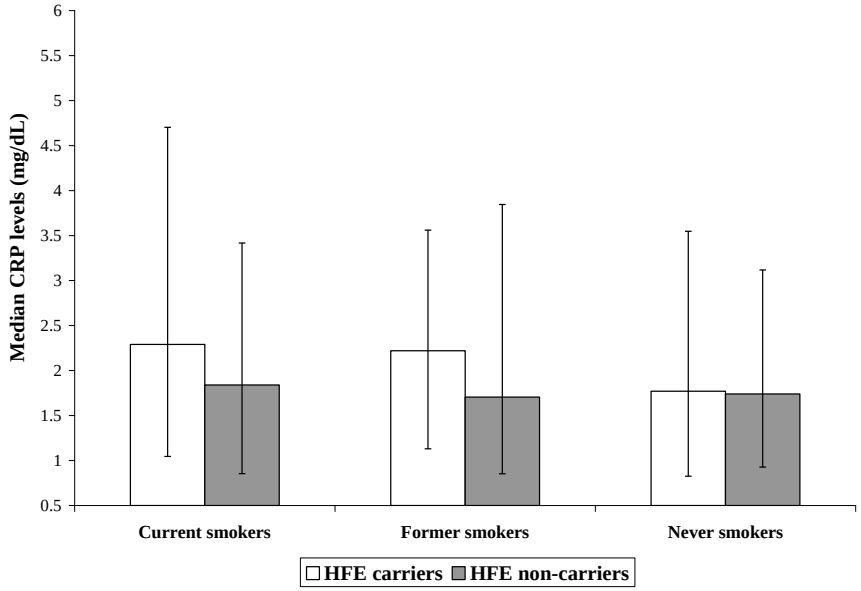
Table 4. **Incidence rates and hazard ratios for incident coronary heart disease by sex, smoking status and *HFE* carrier status**

		CHD	Follow-up (py)	Incidence rate (CHD/1000 py)	Hazard ratio (95% CI) Crude	Adjusted*	Adjusted*
Women							
Never smokers	Non-carriers	50	4.817	10.4 (7.9 to 13.7)	reference	reference	reference
	HFE-carriers	37	2.938	12.6 (9.1 to 17.4)	1.3 (0.9 to 2.0)	1.8 (1.1 to 2.8)	1.8 (1.1 to 2.8)
Former smokers	Non-carriers	33	2.783	11.9 (8.4 to 16.7)	1.6 (1.0 to 2.5)	1.9 (1.1 to 3.1)	reference
	HFE-carriers	20	1.584	12.6 (8.1 to 19.6)	1.7 (1.0 to 3.0)	2.2 (1.2 to 4.0)	1.3 (0.7 to 2.4)
Current smokers	Non-carriers	21	2.05	10.2 (6.7 to 15.7)	1.6 (1.0 to 2.8)	1.4 (0.8 to 2.7)	reference
	HFE-carriers	16	848	18.9 (11.6 to 30.8)	3.1 (1.7 to 5.6)	3.9 (2.1 to 7.3)	3.1 (1.4 to 7.1)
Men							
Never smokers	Non-carriers	9	477	18.9 (9.8 to 36.3)	reference	reference	reference
	HFE-carriers	8	440	18.2 (9.1 to 36.3)	0.9 (0.3 to 2.3)	0.6 (0.2 to 1.8)	0.6 (0.2 to 1.8)
Former smokers	Non-carriers	132	4.801	27.5 (23.2 to 32.6)	1.4 (0.7 to 2.8)	1.2 (0.6 to 2.5)	reference
	HFE-carriers	67	2.902	23.1 (18.2 to 29.3)	1.2 (0.6 to 2.4)	1.0 (0.5 to 2.0)	0.8 (0.6 to 1.1)
Current smokers	Non-carriers	49	2.081	23.5 (17.8 to 31.2)	1.2 (0.6 to 2.5)	1.1 (0.5 to 2.2)	reference
	HFE-carriers	41	1.392	29.4 (21.7 to 40.0)	1.5 (0.7 to 3.2)	1.3 (0.6 to 2.8)	1.2 (0.8 to 1.9)

* Adjusted for body mass index, hypertension, diabetes mellitus, total and high density lipoprotein cholesterol, C-reactive protein and myocardial infarction at baseline. CHD: Coronary heart disease, py: person-years, CI: confidence interval.

Figure 1. **Median C-reactive protein (CRP) levels and interquartile range by *HFE* genotype and smoking status for men and women**

A. Women



B. Men