Published in final edited form as:
Transl Res. 2008 July ; 152(1): 3–10. doi:10.1016/j.trsl.2008.05.005.

HFE C282Y homozygotes have reduced LDL cholesterol: the Atherosclerosis Risk in Communities (ARIC) Study

James S. Pankow[1], **Eric Boerwinkle**[2], **Paul C. Adams**[3], **Eliseo Guallar**[4], **Catherine Leiendecker-Foster**[1], **Jason Rogowski**[1], and **John H. Eckfeldt**[1]

1*University of Minnesota, Minneapolis, MN*

2*University of Texas Health Science Center, Houston, TX*

3*London Health Sciences Centre, London, ON*

4*Johns Hopkins University, Baltimore, MD*

Abstract

Recent studies have raised questions about the long-term health risks for individuals with mutations in the *HFE* gene, although previous studies may have been plagued by selection bias or lack of population-based comparison groups. We examined cardiovascular disease risk factors, iron and liver biomarkers, and morbidity and mortality associated with the C282Y and H63D variants of *HFE* in the ARIC study, a population-based cohort of nearly 16,000 U.S. white and black men who were 45–64 years old at baseline. Subjects were followed for an average of 15 years for death, incident coronary heart disease, stroke, and heart failure, and 8 years for incident diabetes. The prevalence of C282Y homozygosity was 0.42% (45/10,800) in whites, similar to other North American population-based studies. C282Y homozygotes had statistically significantly lower mean LDL cholesterol and fibrinogen and higher mean levels of iron (ferritin, transferrin saturation) and liver biomarkers (alanine aminotransferase, Hepascore) compared to *HFE* wild-type subjects. Rates of all-cause mortality, cardiovascular disease, and diabetes were similar across *HFE* genotypes. These prospective, population-based data indicate higher serum iron indices and possible mild liver dysfunction or disease in some C282Y homozygotes, but provide little evidence that *HFE* C282Y or H63D mutations are related to all-cause mortality, cardiovascular disease, or diabetes. Reduced LDL in C282Y homozygotes may be due to effect of excess iron on cholesterol metabolism and lipoprotein formation in the liver.

Introduction

Hereditary hemochromatosis is a genetic disorder that may lead to excessive iron accumulation in various body tissues. Shortly after the discovery in 1996 that homozygosity for the 845 G->A [C282Y] mutation in the hemochromatosis gene (*HFE)* was the principal cause of hereditary hemochromatosis in individuals of northern European descent [1], it was assumed that most C282Y homozygotes would eventually develop excessive body iron deposition and clinically overt disease, most notably in the liver, heart, pancreas, joints, or pituitary [2]. However, large screening studies from Norway [3] and southern California [4] showed that

Correspondence and reprints: James S. Pankow, Division of Epidemiology and Community Health, University of Minnesota, 1300 South Second Street, Suite 300, Minneapolis, MN 55454, Phone: 612-624-2883, Fax: 612-624-0315, E-mail: pankow@umn.edu.

the prevalence of clinical disease in target organs was similar in C282Y homozygotes compared to subjects without the mutation, and none of the 23 homozygotes in another population-based developed clinical signs or symptoms suggesting hereditary hemochromatosis during 25 years of follow-up [5]. Some of these studies have been criticized for a failure to ascertain subclinical disease, such as liver fibrosis [6]. Further concerns have been raised about the possibility of selection bias, as individuals who already had clinical manifestations were unlikely to be screened or may have died prematurely [7], although at least in some studies, evaluation of Hardy-Weinberg equilibrium found no deficit in the anticipated number of C282Y homozygotes [8].

Although it is unlikely that the vast majority of C282Y homozygotes eventually develop serious morbidity and early mortality, as was suggested less than a decade ago [2], the clinical significance of *HFE* mutations remains uncertain [9]. The U.S. Preventive Services Task Force recently concluded that there is “insufficient evidence to confidently project the impact of, or estimate the benefit from, widespread or high-risk genetic screening for hereditary hemochromatosis [9].” Because health benefits of screening for a disease depend largely on the natural history, determining the incidence of clinically significant morbidity or mortality related to *HFE* mutations is highly relevant. In the present report, we evaluated morbidity and mortality associated with the C282Y and H63D mutations of *HFE* in a U.S. cohort ascertained using carefully designed and executed population-based sampling techniques.

Methods

Study population

In 1987–89 the Atherosclerosis Risk in Communities (ARIC) Study enrolled 15,792 subjects ages 45–64 years in four U.S. locations: Forsyth County, NC; Jackson, MS; seven northwestern suburbs of Minneapolis, MN; and Washington County, MD [10]. Black residents were over-sampled as part of the area-probability sampling of Forsyth County, NC, while enrollment at the Jackson, MS, site was restricted to black residents. Eligible residents were invited to participate in a baseline clinical examination (visit 1) and three subsequent follow-up examinations approximately three years apart (visits 2–4). For the present analysis, we excluded 48 participants who were not white or black, 43 who did not provide consent for genetic studies, and 1,216 with missing data for either the C282Y or H63D variants, leaving 14,485 subjects (10,800 whites and 3685 blacks) available for analysis. The research was carried out according to the principles of the Declaration of Helsinki. Institutional review boards at each study institution provided approval and all participants gave informed consent.

Baseline data collection

At baseline, interviewers collected information on menopausal status, smoking status, and previous diagnoses, including cancer [10]. Fasting lipids, glucose, insulin, and fibrinogen were measured at a central laboratory using methods described previously [11–13], and hematology was performed at local laboratories. Prevalent coronary heart disease was defined as a self-reported history of a physician-diagnosed heart attack, evidence of an old myocardial infarction (MI) by electrocardiogram based on the Minnesota codes, or reported history of coronary revascularization. Prevalent stroke was defined as a reported stroke diagnosed by a physician.

Prevalent diabetes was defined as reported physician diagnosis, use of anti-diabetes medications, fasting (≥ 8hr post prandial) serum glucose ≥ 7 mmol/L (140 mg/dL), or non-fasting glucose of ≥ 11.1 mmol/L (200 mg/dL). Prevalent heart failure was defined as reported current intake of heart failure medication at visit 1 or evidence of manifest heart failure defined by the Gothenburg criteria stage 3 [14,15]. Carotid intimal-medial thickness was measured by B-mode ultrasound [16,17] and the mean intimal-medial thickness across three segments

(internal carotid, bifurcation, common carotid) was used in analysis. History of fatigue was assessed at visit 2 by the question "Do you often feel tired?" History of arthritis was assessed at visit 4 by the question "Has a doctor ever told you that you have arthritis?" *HFE* variants were typed at the ARIC DNA Laboratory, University of Texas Health Sciences Center at Houston using a standard Taqman method. All C282Y homozgyotes (C282Y/C282Y) were verified at CLIA-certified Clinical Laboratories of the University of Minnesota Medical Center, Fairview using a restriction endonuclease method [1].

Iron and liver biomarkers

Because of limited resources, iron and liver biomarkers were measured only in C282Y homozygotes and a comparison group of 48 wild-type subjects randomly selected from white ARIC controls in the Longitudinal Investigation of Thromboembolism Etiology study [18], frequency matched to C282Y homozygotes by age group (45–54, 55–64, or 65–74 years) at the time of venipuncture. When available, serum from the most recent full cohort exam (visit 4, 1996–98) was used; serum from visit 2 (n=8) or visit 1 (n=3) was used for a few subjects, and was unavailable for all visits in one C282Y homozygote. Transferrin saturation was measured using automated spectrophotometric measurement of iron and unsaturated iron binding capacity on a Roche/Hitachi 911 analyzer using commercially available reagents (Roche Diagnostics/Boehringer Mannheim Corp., Indianapolis, IN). Ferritin, alanine aminotransferase, γ-glutamyltransferase, and total bilirubin were measured using the same equipment and commercially available assays from Roche Diagnostics. Alpha-2-macroglobulin was assayed using the Immage analyzer and commercially available reagents (Beckman-Coulter, Inc., Brea, CA), and hyaluronic acid was measured using an ELISA assay kit (Corgenix Inc., Westminster, CO). Coefficients of variation for all assays were less than 5.4% except ferritin and unsaturated iron binding capacity, which were 9.9% and 8.4%, respectively. To assess liver fibrosis, we used a predictive model (Hepascore) based on bilirubin, γ-glutamyltransferase, hyaluronic acid, α_2-macroglobulin, age, and sex previously validated in subjects with chronic hepatitis C infection [19].

Morbidity and mortality follow-up

Liver disease was assessed at visit 3 by the question "Has a doctor ever said you had cirrhosis or another chronic liver disease?" and searches of underlying and contributing causes of death (ICD-9 570–573 or 155; ICD-10 K7 or C22) and hospital discharge diagnoses (ICD-9 CM 570–573 or 155). Clinical diagnosis of hemochromatosis was ascertained as a discharge diagnosis of hemochromatosis (ICD-9 CM 275.0). All-cause mortality was ascertained by reviews of death certificates, annual follow-up interviews, hospital charts, and other means. Details on quality assurance for ascertainment and classification of coronary heart disease (CHD) and stroke events have been published elsewhere [20–22]. Incident CHD events were defined as a validated definite or probable hospitalized MI, definite CHD death, unrecognized myocardial infarction defined by ARIC electrocardiogram readings, or coronary revascularization. Incident ischemic stroke was defined as validated definite or probable hospitalized embolic or thrombotic brain infarctions. Incident heart failure was defined as a hospital discharge diagnosis coded with heart failure at any position (ICD-9 CM 428) or death with heart failure coded as the underlying cause of death (ICD-9 428; ICD-10 I50) [23]. Subjects were defined as having incident diabetes if they met any of the criteria listed above for prevalent diabetes at any of the follow-up visits with date of diagnosis interpolated between visits [24].

Statistical analysis

We tested the C282Y and H63D variants separately for Hardy Weinberg equilibrium using χ^2 goodness of fit tests. Pairwise differences in baseline characteristics between each combined

C282Y and H63D genotype group and a common reference group of subjects wild-type for both variants were tested by analysis of variance (continuous measures) or logistic regression (dichotomous measures) as implemented in SAS (version 9.1, SAS Institute Inc., Cary, NC). For iron and liver biomarkers, differences between C282Y homozygotes and wild-type subjects were tested by analysis of covariance, adjusting for age (matching variable) and sex. Triglycerides, insulin, ferritin, bilirubin, γ-glutamyltransferase, hyaluronic acid, and alanine aminotransferase were log-transformed before analysis. For longitudinal outcomes, we calculated person-years at risk for each participant as the time between the baseline examination date and the last date of follow-up (e.g., December 31, 2004 for all outcomes except diabetes), date of loss-to-follow-up, date of death, or date of disease diagnosis. We used the Epitab procedure in STATA (release 8, StataCorp, College Station, TX) to estimate rate ratios and exact confidence intervals.

Results

Genotype frequencies for C282Y and H63D did not deviate statistically from those expected under Hardy-Weinberg equilibrium in whites or blacks ($p>0.05$). Among whites, there were slightly more C282Y homozygotes (n=45) than expected under Hardy-Weinberg equilibrium conditions (n=43.3). Only four black subjects were either C282Y homozygotes or C282Y/H63D compound heterozygotes (Table 1), thereby precluding detailed analyses of those genotypes. All further analyses were restricted to whites.

Mean LDL cholesterol and fibrinogen levels at baseline were substantially lower among C282Y homozygotes compared to wild-type subjects (Table 2). Similar patterns were observed for LDL during follow-up: mean levels were 0.31 mmol/L (12 mg/dL; $p=0.06$), 0.44 mmol/L (17 mg/dL; $p=0.007$), and 0.47 mmol/L (18 mg/dL; $p=0.003$) lower in C282Y homozygotes at visits 2, 3, and 4, respectively. No adjustment was made for lipid-lowering medications in these analyses. At visit 4, self-reported use of lipid-lowering medications was nearly twice as common among wild-type subjects than C282Y homozygotes (16% vs. 9%), although this difference was not statistically significant. At baseline, mean hematocrit and hemoglobin were higher in carriers of the C282Y or H63D mutations compared to wild-type subjects, although for C282Y homozygotes differences were not statistically significant.

Among C282Y homozygotes, 11 (25%) had ferritin levels greater than 1000 μg/L, while none of the 48 age-matched wild-type subjects had levels exceeding this cutpoint. Mean ferritin, transferrin saturation, and total iron were significantly higher while unsaturated and total iron binding capacities were significantly lower in C282Y homozygotes (Table 3). The Hepascore index was significantly higher in C282Y homozygotes ($p=0.003$), although there were only small differences for individual components of the index, including bilirubin, γ-glutamyltransferase, hyaluronic acid, and α_2-macroglobulin.

Mean length of follow-up was 15 years for all-cause mortality, CHD, stroke, and heart failure, and 8 years for diabetes. Rates of all-cause mortality, incident CHD, stroke, heart failure and diabetes were similar across *HFE* genotypes (Table 4). Five C282Y homozygotes (11%) had clinical evidence of liver disease during follow-up, including two subjects who died with an underlying cause of death coded as hepatocellular carcinoma and cholangiocarcinoma, respectively, two subjects with hospital discharge diagnoses indicating liver disease, and one subject who self-reported liver disease at visit 3. Among wild-type subjects, 3% had clinical evidence of liver disease. Prior to any genetic testing as part of this study, four C282Y homozygotes (9%) had a hospital discharge diagnosis of hemochromatosis during follow-up, while only one wild-type subject (0.01%) had such a diagnosis.

Discussion

We found that genotype frequencies for C282Y and H63D in the population-based ARIC cohort were similar to those reported for whites and blacks in the HEIRS study [25], the U.S.-representative NHANES III investigation [26], and a health maintenance organization in southern California [8]. There was no evidence that C282Y homozygotes had been selectively lost from the population by the time subjects were enrolled in the ARIC cohort, similar to the findings of Beutler et al. [8]. Furthermore, our results indicated similar rates of all-cause mortality across all *HFE* genotypes over an average of 15 years of follow-up. Consistent with our results, cross-sectional studies in Finland [27], the Netherlands [28], and France [29] did not find lower frequencies of the C282Y mutation in older age groups, as might be expected if the mutation is an important cause of death. Similarly, the Melbourne Collaborative Cohort Study found that *HFE* genotypes were unrelated to all-cause mortality over 11.4 years of follow-up in 29,676 subjects of northern European ancestry [6].

C282Y homozygotes had a more favorable lipid profile, with mean LDL-cholesterol concentrations lower than wild-type subjects. To our knowledge, no previous studies have reported an association between C282Y homozygosity and LDL cholesterol. Interestingly, one study found that self-reported cholesterol problems were 3 to 5 times more frequent in wild-type subjects compared to C282Y/C282Y subjects [30]. Although this finding was considered likely due to chance [30], our results are consistent with less frequent use of cholesterol-lowering drugs among C282Y homozygotes. The effect of the *HFE* C282Y mutation on mean LDL levels in the ARIC study (0.38 mmol/L or 15 mg/dL) is similar in magnitude to the range of effects on LDL (0.05 to 0.23 mmol/L or 2 to 9 mg/dL per allele) for the eleven most significant single nucleotide polymorphisms, including those near the *APOE/C1/C4, LDLR*, *APOB*, and *PCSK9* genes, in a combined analysis of three recent genome-wide association studies from Europe [31]. However, the frequency of the C282Y/C282Y genotype is only 0.4–0.5% in populations of European ancestry, making it unlikely that these genome-wide association studies of LDL were sufficiently powered to detect an effect of a rare variant such as *HFE*.

Experimental iron overload in rats has been found to lower LDL and raise HDL [32]. Excess iron could affect cholesterol metabolism due to increased intracellular oxidative stress, membrane peroxidation, and altered activity of liver enzymes involved in cholesterol metabolism and lipoprotein formation [32]. Alternatively, differences in cholesterol levels could reflect long-term liver damage due to iron overload in C282Y homozygotes. Among patients with chronic hepatitis C, total cholesterol levels have been found to be 0.31–0.41 mmol/L (12–16 mg/dL) lower, on average, among those with significant liver fibrosis compared to those with no significant fibrosis, possibly due to reduced cholesterol synthesis in more advanced forms of liver disease [33,34].

According to previously published results from the ARIC Study [35], CHD risk was elevated by about 40% for every 1 mmol/L increment in LDL cholesterol. Therefore, the observed difference in LDL concentration between wild-type subjects and C282Y homozygotes (i.e., approximately 0.4 mmol/L) would predict a 16% lower rate of incident CHD among C282Y homozygotes. However, we found did not find a statistically significant association between either *HFE* C282Y or H63D mutations and risk of coronary heart disease, a finding consistent with other prospective studies [36–39]. Even large cohort studies such as our own are unlikely to detect small reductions in CHD risk possibly mediated by lower LDL concentrations because of the relatively small number of C282Y homozygotes available for follow-up. In contrast to our results, an earlier case-cohort analysis from the ARIC study [40] found that C282Y heterozygotes had a significantly increased risk of CHD after adjustment for traditional risk factors (HR=2.7; 95% CI: 1.2–6.1), but our results do not corroborate that previous analysis.

In fully adjusted models identical to those used in the earlier report, hazard ratios for incident CHD in the present analysis were 0.97 (95% CI: 0.63–1.5) for events ascertained through calendar year 1991, the last date of follow-up in the earlier report, and 1.03 (95% CI: 0.88–1.2) when follow-up was extended through 2004. The discrepancy between the earlier report and present analysis is likely due to the fact that C282Y carriers were underrepresented due to random error introduced when only a sample of the cohort was selected for the earlier study. In the earlier report, the frequency of the *HFE* C282Y mutation in the ARIC cohort was estimated to be 6.1% based on a cohort random sample of only 535 subjects [40]; in the present analysis, the frequency of the C282Y mutation was found to be substantially higher (9.7%) in 10,800 white subjects from the full cohort.

Of the six genotype groups, wild-type subjects had the lowest mean hematocrit and hemoglobin values and highest mean platelet count. Similar patterns have been reported previously [41, 42]. As expected, C282Y homozygotes had higher levels of biomarkers of liver dysfunction and disease, including alanine aminotransferase and the Hepascore index. Hepascore provides an area under the curve of 0.76–0.82 for significant biopsy-determined liver fibrosis among patients with chronic hepatitis C infection [19,43,44]. Although only a few subjects had Hepascore values consistent with significant fibrosis, differences between C282Y homozygotes and wild-type subjects may reflect more subtle or early indications of liver damage. Beutler et al. similarly found that plasma collagen IV concentrations, another marker of mild hepatic fibrosis, were significantly higher in homozygotes compared to wild-type subjects [4].

Strengths of the present study include its large sample size, extensive follow-up, and population-based sampling that provides inference to the general population. A systematic review by the U.S. Preventive Services Task Force noted that the many previous studies of *HFE* may have been plagued by selection bias or lack of population-based comparison groups [9]. The ARIC cohort was enrolled nearly ten years before the discovery of *HFE*, making it unlikely subjects had been previously screened for mutations or that their participation was related to knowledge of their genotype. Limitations include the limited number of C282Y homozygotes and small number of controls tested for iron and liver biomarkers, incomplete information on liver disease, limited to self-report, hospital and death records, and an index of liver fibrosis (Hepascore) of uncertain validity in the context of hemochromatosis. Recent studies found that 5–20% of C282Y homozygotes had subclinical disease detected by biopsy, including cirrhosis and hepatic fibrosis [6,45]. In addition, limited information was available regarding clinical diagnosis of hemochromatosis and markers of iron metabolism were only measured once, thereby providing an incomplete history of iron status. Studies of untreated C282Y homozygotes have demonstrated substantial short- and long-term intra-individual variability in serum ferritin and transferrin saturation levels [46,47].

Speculations

In the ARIC study, C282Y homozygotes had significantly higher levels of serum iron measures and biomarkers of liver function, but there was little evidence that *HFE* genotypes are related to all-cause mortality, cardiovascular disease, or diabetes. Reduced LDL in C282Y homozygotes may be due to effect of excess iron on cholesterol metabolism and lipoprotein formation in the liver.

Abbreviations

LDL, low density lipoprotein; HDL, high density lipoprotein; CHD, coronary heart disease.

Acknowledgments

The authors thank the staff and participants of the ARIC Study for their important contributions. The ARIC study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022.

References

1. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996;13:399–408. [PubMed: 8696333]
2. Edwards CQ, Griffen LM, Ajioka RS, Kushner JP. Screening for hemochromatosis: phenotype versus genotype. Semin Hematol 1998;35:72–76. [PubMed: 9460810]
3. Asberg A, Hveem K, Thorstensen K, Ellekjter E, Kannelonning K, Fjosne U, et al. Screening for hemochromatosis: high prevalence and low morbidity in an unselected population of 65,238 persons. Scand J Gastroenterol 2001;36:1108–1115. [PubMed: 11589387]
4. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of 845G--> A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet 2002;359:211–218. [PubMed: 11812557]
5. Andersen RV, Tybjaerg-Hansen A, Appleyard M, Birgens H, Nordestgaard BG. Hemochromatosis mutations in the general population: iron overload progression rate. Blood 2004;103:2914–2919. [PubMed: 15070663]
6. Allen KJ, Gurrin LC, Constantine CC, Osborne NJ, Delatycki MB, Nicoll AJ, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. N Engl J Med 2008;358:221–230. [PubMed: 18199861]
7. Ajioka RS, Kushner JP. Clinical consequences of iron overload in hemochromatosis homozygotes. Blood 2003;101:3351–3353. [PubMed: 12707221]discussion 4–8
8. Beutler E, Felitti V, Koziol JA, Gelbart T. Clinical haemochromatosis in HFE mutation carriers - Authors' reply. Lancet 2002;360:413–414.
9. Whitlock EP, Garlitz BA, Harris EL, Beil TL, Smith PR. Screening for hereditary hemochromatosis: A systematic review for the US Preventive Services Task Force. Annals of Internal Medicine 2006;145:209–223. [PubMed: 16880463]
10. The ARIC investigators. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. Am J Epidemiol 1989;129:687–702. [PubMed: 2646917]
11. Sharrett AR, Patsch W, Sorlie PD, Heiss G, Bond MG, Davis CE. Associations of lipoprotein cholesterols, apolipoproteins A-I and B, and triglycerides with carotid atherosclerosis and coronary heart disease. The Atherosclerosis Risk in Communities (ARIC) Study. Arterioscler Thromb 1994;14:1098–1104. [PubMed: 8018665]
12. Papp AC, Hatzakis H, Bracey A, Wu KK. ARIC hemostasis study--I. Development of a blood collection and processing system suitable for multicenter hemostatic studies. Thromb Haemost 1989;61:15–19. [PubMed: 2526384]
13. Eckfeldt JH, Chambless LE, Shen YL. Short-term, within-person variability in clinical chemistry test results. Experience from the Atherosclerosis Risk in Communities Study. Arch Pathol Lab Med 1994;118:496–500. [PubMed: 8192558]
14. Wilhelmsen L, Eriksson H, Svardsudd K, Caidahl K. Improving the detection and diagnosis of congestive heart failure. Eur Heart J 1989;10:13–18. [PubMed: 2806286]
15. Eriksson H, Caidahl K, Larsson B, Ohlson LO, Welin L, Wilhelmsen L, et al. Cardiac and pulmonary causes of dyspnoea--validation of a scoring test for clinical-epidemiological use: the Study of Men Born in 1913. Eur Heart J 1987;8:1007–1014. [PubMed: 3665952]
16. Riley WA, Barnes RW, Bond MG, Evans GW, Chambless LE, Heiss G. High-resolution B-mode ultrasound reading methods in the Atherosclerosis Risk in Communities (ARIC) cohort. The ARIC Study Group. J Neuroimaging 1991;1:168–172. [PubMed: 10149810]
17. Bond MG, Barnes RW, Riley WA, Wilmoth SK, Chambless LE, Howard G, et al. High-resolution B-mode ultrasound scanning methods in the Atherosclerosis Risk in Communities Study (ARIC). The ARIC Study Group. J Neuroimaging 1991;1:68–73. [PubMed: 10149803]

18. Folsom AR, Cushman M, Tsai MY, Aleksic N, Heckbert SR, Boland LL, et al. A prospective study of venous thromboembolism in relation to factor V Leiden and related factors. Blood 2002;99:2720–2725. [PubMed: 11929758]
19. Adams LA, Bulsara M, Rossi E, Deboer B, Speers D, George J, et al. Hepascore: An accurate validated predictor of liver fibrosis in chronic hepatitis C infection. Clinical Chemistry 2005;51:1867–1873. [PubMed: 16055434]
20. White AD, Folsom AR, Chambless LE, Sharret AR, Yang K, Conwill D, et al. Community surveillance of coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) Study: methods and initial two years' experience. J Clin Epidemiol 1996;49:223–233. [PubMed: 8606324]
21. ARIC Investigators. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. Am J Epidemiol 1989;129:687–702. [PubMed: 2646917]
22. Rosamond WD, Folsom AR, Chambless LE, Wang CH, McGovern PG, Howard G, et al. Stroke incidence and survival among middle-aged adults: 9-year follow- up of the Atherosclerosis Risk in Communities (ARIC) cohort. Stroke 1999;30:736–743. [PubMed: 10187871]
23. Kottgen A, Russell SD, Loehr LR, Crainiceanu CM, Rosamond WD, Chang PP, et al. Reduced kidney function as a risk factor for incident heart failure: the atherosclerosis risk in communities (ARIC) study. J Am Soc Nephrol 2007;18:1307–1315. [PubMed: 17344421]
24. Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, et al. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes 2003;52:1799–1805. [PubMed: 12829649]
25. Adams PC, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, et al. Hemochromatosis and iron-overload screening in a racially diverse population. N Engl J Med 2005;352:1769–1778. [PubMed: 15858186]
26. Steinberg KK, Cogswell ME, Chang JC, Caudill SP, McQuillan GM, Bowman BA, et al. Prevalence of C282Y and H63D mutations in the hemochromatosis (HFE) gene in the United States. Jama 2001;285:2216–2222. [PubMed: 11325323]
27. Piippo K, Louhija J, Tilvis R, Kontula K. You may live to the age of more than 100 years even if you are homozygous for a haemochromatosis gene mutation. European Journal of Clinical Investigation 2003;33:830–831. [PubMed: 12925044]
28. van Aken MO, de Craen AJM, Gussekloo J, Moghaddam PH, Vandenbroucke JP, Heijmans BT, et al. 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. European Journal of Clinical Investigation 2002;32:750–754. [PubMed: 12406023]
29. Coppin H, Bensaid M, Fruchon S, Borot N, Blanche H, Roth MP. Longevity and carrying the C282Y mutation for haemochromatosis on the HFE gene: case control study of 492 French centenarians. British Medical Journal 2003;327:132–133. [PubMed: 12869454]
30. Waalen J, Beutler E. Beware of multiple comparisons: A study of symptoms associated with mutations of the HFE hemochromatosis gene. Clinica Chimica Acta 2005;361:128–134.
31. Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, Clarke R, et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet 2008;40:161–169. [PubMed: 18193043]
32. Turbino-Ribeiro SM, Silva ME, Chianca DA Jr, De Paula H, Cardoso LM, Colombari E, et al. Iron overload in hypercholesterolemic rats affects iron homeostasis and serum lipids but not blood pressure. J Nutr 2003;133:15–20. [PubMed: 12514260]
33. Forns X, Ampurdanes S, Llovet JM, Aponte J, Quinto L, Martinez-Bauer E, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 2002;36:986–992. [PubMed: 12297848]
34. Sud A, Hui JM, Farrell GC, Bandara P, Kench JG, Fung C, et al. Improved prediction of fibrosis in chronic hepatitis C using measures of insulin resistance in a probability index. Hepatology 2004;39:1239–1247. [PubMed: 15122752]
35. Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D, et al. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. Circulation 2001;104:1108–1113. [PubMed: 11535564]

36. van der A DL, Peeters PHM, Grobbee DE, Roest M, Marx JJM, Voorbij HM, et al. HFE mutations and risk of coronary heart disease in middle-aged women. European Journal of Clinical Investigation 2006;36:682–690. [PubMed: 16968463]
37. Fox CJ, Cullen DJ, Knuiman MW, Cumpston GN, Divitini ML, Rossi E, et al. Effects of body iron stores and haemochromatosis genotypes on coronary heart disease outcomes in the Busselton health study. Journal of Cardiovascular Risk 2002;9:287–293. [PubMed: 12394323]
38. Ellervik C, Tybjaerg-Hansen A, Grande P, Appleyard M, Nordestgaard BG. Hereditary hemochromatosis and risk of ischemic heart disease: a prospective study and a case-control study. Circulation 2005;112:185–193. [PubMed: 15998685]
39. Gunn IR, Maxwell FK, Gaffney D, McMahon AD, Packard CJ. Haemochromatosis gene mutations and risk of coronary heart disease: a west of Scotland coronary prevention study (WOSCOPS) substudy. Heart 2004;90:304–306. [PubMed: 14966054]
40. Rasmussen ML, Folsom AR, Catellier DJ, Tsai MY, Garg U, Eckfeldt JH. A prospective study of coronary heart disease and the hemochromatosis gene (HFE) C282Y mutation: the Atherosclerosis Risk in Communities (ARIC) study. Atherosclerosis 2001;154:739–746. [PubMed: 11257277]
41. Beutler E, Felitti V, Gelbart T, Waalen J. Haematological effects of the C282YHFE mutation in homozygous and heterozygous states among subjects of northern and southern European ancestry. British Journal of Haematology 2003;120:887–893. [PubMed: 12614226]
42. Barton JC, Bertoli LF, Rothenberg BE. Peripheral blood erythrocyte parameters in hemochromatosis: Evidence for increased erythrocyte hemoglobin content. Journal of Laboratory and Clinical Medicine 2000;135:96–104. [PubMed: 10638700]
43. Leroy V, Hilleret MN, Sturm N, Trocme C, Renversez JC, Faure P, et al. Prospective comparison of six non-invasive scores for the diagnosis of liver fibrosis in chronic hepatitis C. J Hepatol 2007;46:775–782. [PubMed: 17321634]
44. Halfon P, Bourliere M, Deydier R, Botta-Fridlund D, Renou C, Tran A, et al. Independent prospective multicenter validation of biochemical markers (fibrotest-actitest) for the prediction of liver fibrosis and activity in patients with chronic hepatitis C: the fibropaca study. Am J Gastroenterol 2006;101:547–555. [PubMed: 16542291]
45. Powell LW, Dixon JL, Ramm GA, Purdie DM, Lincoln DJ, Anderson GJ, et al. Screening for hemochromatosis in asymptomatic subjects with or without a family history. Arch Intern Med 2006;166:294–301. [PubMed: 16476869]
46. Olynyk JK, Hagan SE, Cullen DJ, Beilby J, Whittall DE. Evolution of untreated hereditary hemochromatosis in the Busselton population: a 17-year study. Mayo Clin Proc 2004;79:309–313. [PubMed: 15008603]
47. Adams PC, Reboussin DM, Press RD, Barton JC, Acton RT, Moses GC, et al. Biological variability of transferrin saturation and unsaturated iron-binding capacity. Am J Med 2007;120(999):e1–e7.

Table 1

HFE C282Y and H63D Genotype Frequencies

	C282Y/C282Y	C282Y/H63D	H63D/H63D	C282Y/+	H63D/+	+/+
Whites, N (%)	45 (0.4)	193 (1.8)	257 (2.4)	1084 (10.0)	2453 (22.7)	6768 (62.7)
Blacks, N (%)	1 (0.03)	3 (0.1)	3 (0.1)	100 (2.7)	189 (5.1)	3389 (92.0)

Table 2

Baseline Characteristics (Means (Standard Errors) or Percentages) by *HFE* Genotype, ARIC Whites

Characteristic	C282Y/C282Y (N=45)	C282Y/H63D (N=193)	H63D/H63D (N=257)	C282Y/+ (N=1084)	H63D/+ (N=2453)	+/+ (N=6768)
Age (years)	53.4 (0.9)	54.1 (0.4)	54.5 (0.4)	54.3 (0.2)	54.4 (0.1)	54.4 (0.1)
Gender (% men)	47	**40**	49	45	48	48
Post-menopausal, women (%)	55	66	72	63	65	66
Current smoking (%)	18	30	25	25	25	25
Body mass index (kg/m^2)	26.5 (0.7)	27.5 (0.3)	27.3 (0.3)	27.1 (0.1)	27.1 (0.1)	26.9 (0.1)
LDL cholesterol (mmol/L)	**3.18** (0.15)	3.58 (0.07)	3.59 (0.06)	3.52 (0.03)	3.56 (0.02)	3.56 (0.01)
HDL cholesterol (mmol/L)	1.41 (0.06)	1.30 (0.03)	1.28 (0.03)	1.31 (0.01)	1.30 (0.01)	1.31 (0.01)
Triglycerides (mmol/L)†	1.18 (0.80–1.94)	1.37 (0.98–1.91)	**1.39** (1.05–1.94)	1.33 (0.93–1.88)	1.31 (0.93–1.88)	1.28 (0.93–1.83)
Fasting glucose (mmol/L)‡	5.46 (0.08)	5.46 (0.04)	5.50 (0.03)	5.45 (0.02)	5.48 (0.01)	5.47 (0.01)
Fasting insulin (pmol/L)‡	65.2 (8.4)	75.9 (4.1)	69.2 (3.7)	72.6 (1.7)	73.1 (1.2)	72.4 (0.7)
Fibrinogen (g/L)	**2.79** (0.09)	3.05 (0.04)	3.00 (0.04)	2.97 (0.02)	2.98 (0.01)	2.97 (0.01)
Hematocrit (volume fraction)	0.425 (0.006)	**0.431** (0.003)	**0.426** (0.002)	**0.424** (0.001)	**0.423** (0.001)	0.421 (0.001)
Hemoglobin (mmol/L)	2.218 (0.030)	**2.250** (0.015)	**2.220** (0.013)	**2.205** (0.006)	**2.197** (0.004)	2.182 (0.002)
White cell count (10^9 cells/L)	6.02 (0.30)	6.52 (0.14)	6.48 (0.13)	6.27 (0.06)	**6.38** (0.04)	6.27 (0.02)
Platelet count (10^9 cells/L)	243 (11)	252 (6)	256 (5)	257 (2)	256 (2)	260 (1)
Hypertension (%)	22	**33**	**34**	28	**29**	26
Carotid IMT (mm)	0.724 (0.031)	0.716 (0.015)	0.719 (0.013)	0.722 (0.006)	0.725 (0.004)	0.727 (0.002)
History of cancer (%)	9.1	5.7	7.4	**5.1**	6.5	7.2
History of fatigue (%)§	40	49	47	45	45	43
History of arthritis (%)//	29	32	42	41	40	38

IMT: intimal-medial thickness

estimates listed in bold are significantly different ($p<0.05$) compared to +/+ subjects

† medians and interquartile ranges are shown

‡ among subjects without diabetes

§ measured at visit 2

// measured at visit 4

Table 3

Serum iron and liver biomarkers by *HFE* C282Y Genotype, ARIC Whites

Biomarker	C282Y/C282Y (N=44)		+/+ (N=48)		p-value*
	Median	Interquartile Range	Median	Interquartile Range	
Ferritin (μg/L)†	741	377–1143	157	82–238	<0.001
Transferrin saturation (fraction saturation)	0.77	0.62–0.95	0.30	0.24–0.35	<0.001
Total iron (μmol/L)	33.8	27.4–38.3	16.1	12.9–19.9	<0.001
Unsaturated iron binding capacity (μmol/L)	10.9	2.0–18.1	40.1	33.5–45.5	<0.001
Total iron binding capacity (μmol/L)	43.7	38.8–50.8	55.8	51.7–61.4	<0.001
Hepascore†	0.10	0.06–0.26	0.08	0.06–0.12	0.003
Bilirubin (μmol/L)†	6.8	5.1–10.3	6.8	5.1–7.7	0.15
Gamma glutamyltransferase (U/L)†	20	15–30	20	12–31	0.71
Hyaluronic acid (μg/L)†	24	15–34	19	13–27	0.05
α_2-macroglobulin (g/L)	1.9	1.6–2.3	1.8	1.6–2.2	0.09
Alanine aminotransferase (U/L)†	20	14–29	11	8–14	<0.001

*obtained from linear regression models adjusted for age and sex

†log-transformed for linear regression analysis

Table 4

All-Cause Mortality and Incident Disease by *HFE* Genotype, ARIC Whites

Outcome		C282Y/C282Y	C282Y/H63D	H63D/H63D	C282Y/+	H63D/+	+/+
All-Cause Mortality	**Deaths**	7	30	45	174	399	1066
	Person-years	704	2,954	3,900	16,717	37,599	103,755
	Rate per 1000 person-years	9.9	10.2	11.5	10.4	10.6	10.3
	Rate ratio (Exact 95% CI)	0.97 (0.39–2.00)	0.99 (0.66–1.42)	1.12 (0.81–1.51)	1.01 (0.86–1.19)	1.03 (0.92–1.16)	1.00 (ref)
Incident CHD	**Cases**	6	28	31	145	308	860
	Person-years	670	2,638	3,463	14,820	33,191	92,218
	Rate per 1000 person-years	9.0	10.6	9.0	9.8	9.3	9.3
	Rate ratio (Exact 95% CI)	0.96 (0.35–2.10)	1.14 (0.75–1.66)	0.96 (0.65–1.37)	1.05 (0.87–1.25)	1.00 (0.87–1.13)	1.00 (ref)
Incident Stroke	**Cases**	1	11	11	50	93	245
	Person-years	692	2,887	3,799	16,215	36,680	101,316
	Rate per 1000 person-years	1.4	3.8	2.9	3.1	2.5	2.4
	Rate ratio (Exact 95% CI)	0.60 (0.01–3.36)	1.58 (0.78–2.89)	1.20 (0.59–2.18)	1.27 (0.92–1.73)	1.05 (0.82–1.34)	1.00 (ref)
Incident Heart Failure	**Cases**	2	16	23	100	224	564
	Person-years	704	2,697	3,658	15,384	34,934	96,001
	Rate per 1000 person-years	2.8	5.9	6.3	6.5	6.4	5.9
	Rate ratio (Exact 95% CI)	0.48 (0.06–1.75)	1.01 (0.57–1.65)	1.07 (0.67–1.62)	1.11 (0.89–1.37)	1.09 (0.93–1.28)	1.00 (ref)
Incident Diabetes	**Cases**	3	19	20	87	241	566
	Person-years	299	1,312	1,583	7,400	16,397	45,805
	Rate per 1000 person-years	10.0	14.5	12.6	11.8	14.7	12.4
	Rate ratio (Exact 95% CI)	0.81 (0.17–2.39)	1.17 (0.70–1.85)	1.02 (0.62–1.59)	0.95 (0.75–1.19)	1.19 (1.02–1.39)	1.00 (ref)