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HFE C282Y Homozygosity Is Associated With Lower Total and Low-Density Lipoprotein Cholesterol

The Hemochromatosis and Iron Overload Screening Study

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Background—Previous studies have suggested a positive association of coronary heart disease risk and both serum ferritin concentrations and C282Y heterozygosity. Relationships between serum lipids, C282Y homozygosity, and serum ferritin have not been well established.

Methods and Results—The Hemochromatosis and Iron Overload Screening study screened 101 168 participants in primary care from 5 field centers in the United States and Canada with serum ferritin, transferrin saturation, and *HFE* genotyping for C282Y and H63D mutations. Serum lipids were measured in a subset of 176 C282Y homozygotes (63 male, 113 female whites) without a prior diagnosis of, family history, or treatment for hemochromatosis and a matched sample of participants with normal transferrin saturation and serum ferritin without C282Y or H63D mutations (wild-type, 123 male, 189 female whites). The proportion of subjects who reported using prescription cholesterol-lowering medications was ≈3 times higher in *HFE* wild-type subjects than C282Y homozygotes among men (22% versus 7%; $P=0.02$) and, in women, 2 times higher (16% versus 8%; $P=0.07$). After excluding subjects taking cholesterol medications, C282Y homozygotes had significantly lower mean total and low-density lipoprotein cholesterol concentrations than wild-type subjects, with larger genotypic differences for low-density lipoprotein in men (−0.62 mmol/L; 95% CI, −0.93 to −0.33) than in women (−0.28 mmol/L; 95%, CI −0.52 to −0.08).

Conclusions—Total mean serum cholesterol and low-density lipoprotein levels were lower in C282Y homozygotes than in *HFE* wild-type participants. Further studies are required to determine whether this is related to iron overload, *HFE* alleles, or other factors on C282Y-positive chromosome 6p haplotypes. **(*Circ Cardiovasc Genet*. 2009;2:34-37.)**

Key Words: hemochromatosis ■ iron overload ■ iron

Hemochromatosis associated with homozygosity for the C282Y mutation of the *HFE* gene is the most common genetic disorder in white populations with a prevalence of ≈1 in 200 to 300 in white persons of northern European descent.[1] It has been suggested that there may be a biological advantage to persons carrying mutations of the *HFE* gene.[2] Population studies of elderly subjects have demonstrated no difference in the frequency of *HFE* mutations compared to younger subjects, suggesting minimal effects of these mutations on longevity.[3,4] There have been conflicting reports of the association of iron, serum ferritin (SF), and *HFE* mutations with coronary heart disease (CHD).[5–13] Most studies have reported on associations with C282Y heterozygotes, a group which is very unlikely to have significant iron overload.[1] A previous carefully designed population-based study reported lower total cholesterol and low-density lipoprotein (LDL) cholesterol in 48 C282Y homozygotes compared with *HFE* wild-type subjects.[14] In the current study, the relationship between serum lipids, SF, and transferrin saturation (TS) in C282Y homozygotes is reported in a subset of participants from the Hemochromatosis and Iron Overload Screening (HEIRS) study.[1]

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Methods

The authors had full access to and take full responsibility for the integrity of the data. We have read and agree to the manuscript as written. The multicenter, multiethnic, primary care–based HEIRS study performed initial screening after informed consent on 101 168 participants for hemochromatosis and iron overload using TS and SF measurements and HFE genotyping (C282Y and H63D alleles).[1]

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Table 1. Adjusted* Means or Percentages of Iron, Liver, and Lipid Measures by *HFE* Genotype

	Men			Women					
Measure	C282Y/C282Y (n=63)	+/+ (n=123)	Difference or Ratio (95% CI)	C282Y/C282Y (n=113)	+/+ (n=189)	Difference or Ratio (95% CI)	P_{men}	P_{women}	$P_{interaction}$†
Transferrin saturation, %	73	32	41 (36 to 46)	64	26	38 (34 to 42)	<0.001	<0.001	0.11
Ferritin, μg/L	1036	135	901 (730 to 1072)	380	67	313 (235 to 392)	<0.001	<0.001	<0.001
ALT, U/L	36	26	10 (3 to 16)	20	23	−3 (−7 to 2)	<0.001	0.24	<0.001
AST, U/L	39	34	4 (0 to 9)	22	24	−2 (−8 to 2)	0.02	0.33	0.07
GGT, U/L	37	35	2 (−9 to 14)	26	24	2 (−6 to 9)	0.54	0.45	0.99
History of liver disease, %	15	5	2.8 (1.1 to 7.3)	8	7	1.1 (0.49 to 2.7)	0.03	0.75	0.32
Lipid-lowering medication, %	7	22	0.30 (0.11 to 0.83)	8	16	0.51 (0.25 to 1.1)	0.02	0.07	0.57
Total cholesterol, mmol/L‡	4.91	5.56	−0.65 (−1.01 to −0.28)	5.28	5.51	−0.23 (−0.49 to 0)	<0.001	0.05	0.27
LDL cholesterol, mmol/L‡	2.79	3.41	−0.62 (−0.93 to −0.33)	3.00	3.28	−0.28 (−0.52 to −0.08)	<0.001	0.01	0.26
HDL cholesterol, mmol/L‡	1.22	1.22	0 (−0.10 to 0.08)	1.47	1.50	−0.03 (−0.13 to 0.05)	0.93	0.39	0.61
Triglycerides, mmol/L‡	2.15	2.23	−0.08 (−0.88 to 0.72)	1.75	1.69	0.06 (−0.21 to 0.31)	0.84	0.70	0.75

1 mmol/L of total, LDL, and HDL cholesterol=38.67 mg/dL.

ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; HDL, high-density lipoprotein.

*Adjusted for age and field center.

†Test of homogeneity across genders in difference in mean or prevalence ratio.

‡Excluding subjects taking lipid-lowering medications.

All C282Y homozygotes identified in the HEIRS study were invited to participate in a postscreening clinical examination that included collection of blood samples for additional testing. In addition, a frequency-matched comparison group was invited to attend the clinical examination from initial screening participants who had the following: (1) *HFE* genotype wt/wt (wild type); and (2) both TS and SF in the eligible range. Eligible ranges for men were TS 20% to 34% and SF 87 to 247 μg/L. Eligible ranges for women were TS 16% to 28% and SF 19 to 121 μg/L. Variables used for the frequency matching were field center (University of Alabama at Birmingham, University of California at Irvine, Howard University, Kaiser Permanente–Portland and Hawaii, London Health Sciences Centre), age group (24 to 44, 45 to 64, ≥65 years), and date of initial screening visit. Almost all C282Y homozygotes in the HEIRS study were white. Therefore, we restricted the present analysis to subjects who reported that they were white. Medical history including medications was recorded by the examining physician or nurse practitioner.

Total serum cholesterol was measured using the Roche cholesterol oxidase method with the Roche Modular P Chemistry analyzer (Roche Diagnostics Corporation, Indianapolis, Ind). High-density lipoprotein cholesterol was measured in serum using the high-density lipoprotein cholesterol plus third-generation direct method, and triglycerides were measured with the triglyceride GB reagent using the same analyzer. LDL cholesterol was calculated in serum specimens having a triglyceride value <4.52 mmol/L using the formula of Friedewald.[15] A total of 51 subjects fasting for <12 hours or missing information on fasting status were excluded from analyses of triglycerides and LDL cholesterol. Hypercholesterolemia was defined as total cholesterol of 6.2 mmol/L or greater or current use of cholesterol-lowering medications. Measurement of SF and TS has been previously described.[1]

For continuous variables such as TS, pairwise differences in characteristics between C282Y homozygotes and the comparison group of wild-type subjects were tested by linear regression. For dichotomous variables, such as history of liver disease or hypercholesterolemia, prevalence ratios and 95% CIs were estimated using relative risk regression (binomial regression with a log link). Tests for interaction were obtained by fitting a model that combined men and women and including an interaction term (sex×genotype). All analyses were implemented in SAS (version 9.1, SAS Institute Inc, Cary, NC).

Results

Age and field center adjusted means or percentages of iron, liver, and lipid measures by *HFE* genotype are shown in Table 1. The age range in the HEIRS study was 25 to 100 years, with a median age of 51 years. The use of prescription cholesterol-lowering medications was 22% in *HFE* wild-type men and 16% in *HFE* wild-type women. In C282Y homozygotes, these medications were used significantly less often in men (7%, $P=0.003$) and women (8%, $P=0.05$) than in control subjects. After excluding subjects taking cholesterol medications, C282Y homozygotes had significantly lower mean total and LDL cholesterol concentrations than wild-type subjects, with larger genotypic differences for LDL in men (−0.62 mmol/L; 95% CI, −0.93 to −0.33) than in women (−0.28 mmol/L; 95% CI, −0.52 to −0.08). There was a significant inverse relationship between LDL and SF ($r=-0.12$, $P=0.02$) and TS ($r=-0.25$, $P<0.001$). Among C282Y homozygotes, the correlation between TS and LDL was −0.16 ($P=0.06$; n=137). Among wild-type subjects, the correlation between TS and LDL was 0.01 ($P=0.90$; n=219). Indirect measures of iron status (SF and TS) were markedly higher in both male and female C282Y homozygotes, and there was a statistically significant interaction between ferritin and *HFE* mutation by gender ($P<0.001$; Table 1). Because of this strong association, it was difficult to separate the potential contributions of *HFE* genotype and iron measures to LDL cholesterol levels. LDL cholesterol levels of C282Y homozygotes and wild-type subjects were compared according to TS (Figure). In men, age and center-adjusted prevalence of

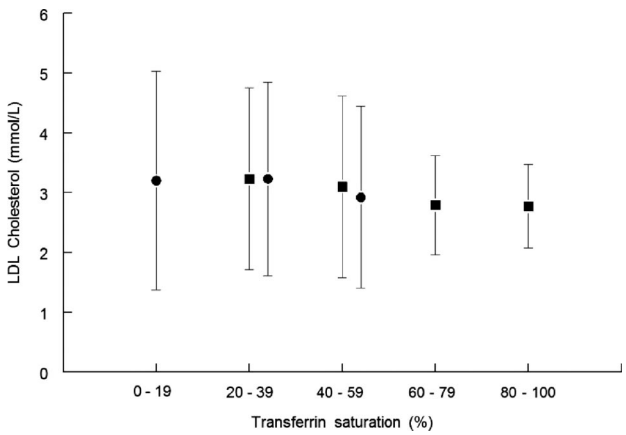

Figure. LDL cholesterol in C282Y homozygotes (■) compared with control participants (●), categorized by serum transferrin saturation. Data are expressed as mean with 95% CIs.

Table 2. Association of *HFE* Genotype and Hypercholesterolemia

		Men		Women	
Model	Covariates	Prevalence Ratio*	95% CI	Prevalence Ratio*	95% CI
1	Age and center	0.39	0.20–0.75	0.76	0.54–1.08
2	Model 1 variables+history of liver disease	0.38	0.20–0.73	0.79	0.56–1.12
3	Model 1 variables+ALT, AST, and GGT	0.43	0.22–0.83	0.75	0.53–1.06
4	Model 1 variables+SF and TS	0.81	0.31–2.13	0.79	0.44–1.42

ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase.
*Prevalence of hypercholesterolemia in C282Y homozgyotes vs wild-type homozygotes.

hypercholesterolemia was significantly lower in C282Y homozygotes than in wild-type participants (Table 2). This association remained after additional adjustment for history of liver disease, or aspartate aminotransferase, alanine aminotransferase, and γglutamyl transferase, but was substantially attenuated when adjusted for TS and SF (Table 2). In women, the association between hypercholesterolemia and *HFE* genotype was not statistically significant in any of the regression models (Table 2). We performed age stratification (<55 and ≥ 55) and found the LDL effect was larger in younger subjects (0.44 mmol/L) than in older subjects (0.21 mmol/L). A formal test of genotype×age interaction was not significant.

Discussion

CHD has not been reported commonly in long-term studies of C282Y homozygotes[16,17] despite its being the most common cause of death in most Western countries. In this study, we have determined that C282Y homozygotes have a significantly lower total and LDL cholesterol. The direction and magnitude of the effect are very similar to those in a previous report in a study of 48 C282Y homozygotes in the Atherosclerosis and Risk in Communities study.[14]

A key question is whether the magnitude of the difference in total or LDL cholesterol reported in this study is clinically significant. A meta-analysis of clinical trials predicted that every 10% of cholesterol lowering reduces CHD mortality by 15% and total mortality by 11%.[18] In the HEIRS study, male and female C282Y homozygotes had total cholesterol levels that were 12% and 4% lower, respectively, than their wild-type counterparts. Several large population-based studies have not demonstrated any differences in CHD morbidity or mortality events across all *HFE* genotypes,[12,14] but it is unlikely that these studies were large enough to detect modest reductions in risk for C282Y homozygotes.

Differences in serum cholesterol concentrations could result from long-term liver damage due to iron overload in C282Y homozygotes. It seems unlikely to be related to liver disease with decreased cholesterol synthesis because most participants had relatively mild iron overload. Liver biopsies were infrequently performed in the HEIRS study and cirrhosis was a rare observation.[19] Among patients with chronic hepatitis C, total cholesterol levels were 0.31 to 0.41 mmol/L (12 to 16 mg/dL) lower among those with significant liver fibrosis than in those with no significant fibrosis.[20,21]

It is important to establish whether the observations in this study regarding the relationship of serum iron measures and cholesterol and LDL cholesterol values apply to patients without *HFE* mutations. The magnitude of the differences in cholesterol would have public health significance if they could be extrapolated to the general population. The effects of oral iron supplements on serum lipids have not been clearly established, and many multivitamin preparations that claim to lower cholesterol contain iron. Experimental iron overload in rats has been found to lower LDL and raise high-density lipoprotein.[22] 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.[22] Patients with hemochromatosis typically have low levels of hepcidin that increase ferroportin expression on macrophages, which decrease intracellular iron. Foam cells in the arterial wall are deteriorated macrophages and may have decreased iron and less oxidative damage.[23]

It is important to recognize that elevated SF may be associated with CHD because SF may be a marker of obesity, the metabolic syndrome, diabetes, and inflammation. In this study, the selection of controls with a normal SF reduced the chance of including wild-type participants with metabolic syndrome, and therefore the differences observed here may be more marked than in the general population.

An unresolved question arising from these studies is whether the decreased cholesterol in C282Y homozygotes is related to excess iron, or a genetic effect of the *HFE* gene itself or other genes in close proximity such as those in the HLA region which are in linkage disequilibrium with the *HFE* locus.[24] The HEIRS study design resulted in a subset of C282Y homozygotes with a broad range of SF levels and included nonexpressing patients with a normal SF. Further studies should include the study of serum lipids in C282Y homozygotes before and after phlebotomy therapy. In conclusion, we have confirmed the previous observation, that C282Y homozygotes have a lower total and LDL cholesterol than matched controls without *HFE* mutations. This may be a factor in the reportedly normal life expectancy that has been reported in patients in population studies and an elucidation of the underlying basis for these observations could be relevant to population health.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Total cholesterol and LDL were found to be significantly lower in patients that were homozygous for the C282Y mutation of the hemochromatosis (HFE) gene in the Hemochromatosis and Iron Overload Screening (HEIRS) Study. The HEIRS study was a multi-ethnic North American study that screened 99, 711 participants for iron overload and *HFE* mutations. Mechanisms for this potential inverse association are unknown.