Mutations in the HFE Gene and Cardiovascular Disease Risk

An Individual Patient Data Meta-Analysis of 53 880 Subjects

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Background—Whether mutations in the hemochromatosis (HFE) gene increase cardiovascular disease risk is still undetermined. The main reason is the low frequency of the mutations, in particular of the compound C282Y/H63D genotype. We combined the data of 11 observational studies for an individual patient data meta-analysis.

Methods and Results—Individual patient data were obtained from published as well as unpublished studies that had information available on the C282Y mutation as well as the H63D mutation in relation to coronary heart disease risk. Individual records were provided on each of the 53 880 participants in 11 studies. In total, 10 541 patients with coronary events were documented, of whom 5724 had an acute myocardial infarction. The crude and adjusted association between HFE genotypes and coronary events was examined by logistic regression analysis. We explored potential effect modification of the association between traditional cardiovascular risk factors and coronary events by HFE genotypes. After full adjustment, the odds ratio for coronary heart disease was 1.12 (95% CI, 0.92 to 1.37) for subjects with the compound heterozygous (C282Y/H63D) genotype relative to those with the wild-type/wild-type genotype. The odds ratios for C282Y/C282Y, C282Y/wild-type, H63D/H63D, and H63D/wild-type were 0.78 (95% CI, 0.49 to 1.26), 0.98 (95% CI, 0.90 to 1.07), 1.16 (95% CI, 0.97 to 1.38), and 1.07 (95% CI, 1.00 to 1.14), respectively. There was no evidence for effect modification.

Conclusions—The results of this large individual patient data meta-analysis do not support the view that HFE gene mutations are associated with an increased risk of coronary heart disease or acute myocardial infarction. **(*Circ Cardiovasc Genet*. 2008;1:43-50.)**

Key Words: cardiovascular diseases ■ epidemiology ■ meta-analysis ■ myocardial infarction ■ risk factors

In the early 1980s, Sullivan[1] proposed that an increase in body iron levels and subsequent oxidative stress may play a role in the occurrence of cardiovascular disease. A first report in 1992 that serum ferritin concentrations ≥200 μg/L were associated with a >2-fold increased risk of acute myocardial infarction (AMI) in men[2] fueled much new research from both experimental and epidemiological studies.[3]

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Up until 1996, blood markers of iron status and dietary iron intake were mostly studied as indicators of iron exposure, but from that year on new opportunities for research were opened because of the discovery of the hemochromatosis-related gene (HFE).[4] The 2 most important mutations in the HFE

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Table 1. Characteristics of the Participating Study Populations

Authors	Country	Design	CHD Cases/Controls, n	AMI Cases, n	Frequency 282Y Allele†	Frequency 63D Allele†
Battiloro et al[15]	Italy	Case-control	171/187	171	0.8 (0–1.9)	16.3 (12.8–20.1)
Hetet et al[10]	United Kingdom	Case-control	564/570	564	10.2 (8.5–11.9)	14.8 (12.7–16.9)
	France	Case-control	152/117	152	6.4 (3.4–9.4)	17.5 (13.3–22.2)
Claeys et al[20]	Switzerland	Case-control	177/89	177	5.1 (2.3–8.4)	17.4 (12.4–23.6)
Fox et al[19]	Australia	Cross-sectional	273/2053	173	8.0 (7.2–8.8)	15.1 (14.0–16.2)
Waalen et al[18]	United States	Cross-sectional	3286/27 763	1121	6.3 (6.1–6.5)	14.8 (14.5–15.1)
Candore et al[11]	Italy (north)	Case-control	172/207	172	1.7 (0.7–2.9)	15.7 (12.6–19.1)
	Italy (south)	Case-control	77/172	77	0	12.5 (9.3–15.7)
Campbell et al[21]	United Kingdom	Case-control	1255/493	828	10.7 (8.8–12.6)	15.2 (13.0–17.4)
Gunn et al[22]	United Kingdom	Nested case-control	482/1104	411	8.4 (7.2–9.5)	14.2 (12.7–15.5)
Ellervik et al[12]	Denmark	Prospective+ Case-control	3539/8080	1682	5.5 (5.2–5.9)	12.5 (12.0–13.0)
Van der A et al[8]	Netherlands	Prospective	207/1465	69	5.4 (4.5–6.2)	14.0 (12.8–15.3)
Cobbaert et al[23]*	Netherlands	Prospective	159/1066	127	6.6 (5.5–7.6)	14.1 (12.7–15.7)

*The unpublished study by Cobbaert et al[23] was undertaken within the Monitoring Project on Disease Risk Factors, a large survey of cardiovascular risk factors in the Netherlands.[39]

†Percentages and 95% CIs in control subjects.

gene, 282C→Y and 63H→D, have both been associated with varying degrees of iron overload[5] and could be viewed as a marker of lifelong exposure to increased iron levels.

Despite extensive research, the exact mechanism of the HFE protein in iron metabolism is still elusive. There is evidence that HFE competes with transferrin for binding to the transferrin receptor, thereby lowering its affinity for iron-containing transferrin and downregulating the uptake of iron in the cells.[6,7] The 2 mutations C282Y and H63D appear to be in complete linkage disequilibrium. Whereas the C282Y mutation disrupts the formation of the disulfide bridge necessary for a proper cell-surface expression of the HFE protein, the functionality of H63D is less well established.

Last year, we reported the results of a conventional meta-analysis of HFE genotypes and cardiovascular disease risk using aggregated data, indicating no increased risk.[8] However, definitive conclusions could not be drawn because we could not study the effect of the joint HFE mutations and make a clear distinction between single heterozygous and compound heterozygous subjects. The latter group expresses higher biochemical iron indices than single heterozygotes.[9] Most publications to date, including our own, lack the statistical power for such a comparison and have therefore not presented data for combined genotypes.

To be able to study the effect of the combined C282Y and H63D mutations on the risk of cardiovascular disease, we conducted an individual patient data (IPD) meta-analysis for which we collected all published and (known) unpublished individual data on the association between HFE mutations and coronary heart disease (CHD). In addition, we investigated the possibility of interaction between the HFE gene and classic risk factors for cardiovascular disease.

Methods

Study Selection, Data Collection, and End Points

Studies were identified by searching PubMed from 1996 until August 2007 with the use of the strategy (["Hemochromatosis" {MeSH}] OR "Hemochromatosis" OR "HFE"] AND ["Myocardial infarction" {MeSH} OR "Coronary" OR "Heart disease"]) and hand-searching the reference lists for additional articles. Our search was limited to the English language. From our global search, studies were identified that fulfilled the following criteria: (1) association study in human subjects; (2) genotype data available on both the HFE 282C→Y and 63H→D mutations; and (3) "hard" end points of CHD. Studies on intermediate phenotypes such as intima-media thickness were excluded. We did not limit the search to studies of 1 specific study design but included case-control as well as prospective and cross-sectional studies.

The principal investigators of all eligible studies were asked for the raw data of their studies. The obtained data were thoroughly checked for consistency, plausibility, and integrity of follow-up. Any queries were resolved by the responsible investigator or statistician.

Cardiovascular end points were coded by the investigators according to the *International Classification of Diseases* (*ICD*) version 9 or 10, and if no *ICD* code was available, we asked for a description of the end point as detailed as possible. End points were classified as CHD ([unstable] angina, AMI, acute and chronic ischemia; or *ICD-9* codes 410 to 414, 427.5; 798.1, 798.2, 798.9; or *ICD-10* codes I10 to I25) and AMI (AMI or *ICD-9* code 410 or *ICD-10* codes I21 and I22). Current smoking status was categorized into yes/no. Hypertension was defined as a measured systolic blood pressure >160 mm Hg and/or diastolic blood pressure >95 mm Hg and/or treatment for hypertension. Hypercholesterolemia was defined as fasting total cholesterol ≥5.0 mmol/L or nonfasting total cholesterol ≥8.0 mmol/L. Diabetes was defined as fasting glucose ≥7.0 mmol/L or nonfasting glucose ≥11.1 mmol/L and/or treatment for diabetes. For the exploration of interaction, age was divided into 2 groups based on the median value in the data set.

Before analysis, data were checked and coded in a standard fashion and entered into a common database. All data sets were analyzed as case-control comparisons, including the studies that were by origin prospective or cross-sectional in design. The articles by Hetet et al[10] and Candore et al[11] included 2 different studies, and they were treated as such in our analyses. The article by Ellervik et al[12] was based on a case-control and a prospective study, using 1 and the same control group. We therefore combined the data and analyzed the cases from the case-control study and the cases from the prospective study as 1 group and compared them with the controls.

Table 1. Continued

	HFE Genotype					
HFE Genotype, Total, n (%)	Wild-Type/Wild-Type, n (%)	H63D/Wild-Type, n (%)	H63D/H63D, n (%)	C282Y/Wild-Type, n (%)	C282Y/H63D, n (%)	C282Y/C282Y, n (%)
358 (100)	245 (68)	94 (26)	12 (3)	6 (2)	1 (<1)	…
1134 (100)	626 (55)	242 (21)	31 (3)	176 (16)	36 (3)	23 (2)
269 (100)	154 (57)	74 (28)	4 (1)	32 (12)	5 (2)	…
266 (100)	166 (62)	64 (24)	5 (2)	23 (9)	6 (2)	2 (<1)
2326 (100)	1358 (58)	567 (24)	48 (2)	293 (13)	44 (2)	16 (<1)
31 049 (100)	19 356 (62)	7231 (23)	733 (2)	3039 (10)	551 (2)	139 (<1)
379 (100)	271 (72)	91 (24)	7 (2)	9 (2)	1 (<1)	…
249 (100)	188 (76)	60 (24)	…	1 (<1)	…	…
1748 (100)	985 (56)	411 (24)	42 (2)	242 (14)	53 (3)	15 (<1)
1586 (100)	935 (59)	359 (23)	29 (2)	223 (14)	36 (2)	4 (0)
11 619 (100)	7727 (67)	2400 (21)	202 (2)	1089 (9)	170 (1)	31 (<1)
1672 (100)	1081 (65)	393 (24)	21 (1)	149 (9)	23 (1)	5 (<1)
1225 (100)	770 (63)	268 (22)	31 (3)	131 (11)	21 (2)	4 (<1)

Data Analysis

The PROC ALLELE procedure in the SAS/Genetics package was used to compute allele frequencies for each study and to test genotype frequencies for deviation from Hardy-Weinberg equilibrium with a χ^2 goodness-of-fit test.

To decide whether pooling was justified, heterogeneity between studies was assessed for carriers of the 282Y and 63D allele separately, with the use of the I-square.[13] Because the I-square for both the 282Y allele and 63D allele was lower than 25% (ie, 8.5% and 0%, respectively), pooling was indeed justified and performed. Combined HFE genotypes were attributed to each individual. Our primary hypothesis was to study the effect of HFE genotypes on CHD risk by case-control comparisons using unmatched logistic regression analysis. The minimum detectable odds ratio (OR) was calculated with the use of the statistical program POWER (version 1.30; Epicenter software) by assuming a β of 0.20 (power of 80%) and an α of 0.05 (2-sided). For example, for compound heterozygotes, our sample size would be able to detect a true OR of 1.23 for CHD and 1.30 for AMI. The analyses performed were based on a complete-case analysis including only those subjects for which information was available on all covariates in the model. This yielded some problems when we adjusted our models for classic cardiovascular risk factors such as smoking, diabetes, hypercholesterolemia, and hypertension. The study by Candore et al[11] contained control sets for which information on these risk factors was unavailable. Similarly, in the study by Hetet et al,[10] information on the presence of diabetes was absent. Because of the complete-case analysis, these studies were therefore automatically excluded from the fully adjusted models. For better comparisons, we therefore also presented minimally adjusted models (including study) without these 2 studies.

Sensitivity analyses were performed to assess sensitivity of the ORs to the individual studies by excluding 1 study at a time.

As a secondary hypothesis, we investigated effect modification by modeling the association between several classic cardiovascular risk factors and CHD risk within strata of HFE genotypes (carriage of 0, 1, or 2 mutated alleles). The probability value for multiplicative interaction was based on the likelihood ratio test comparing models with and without interaction terms. Ideally, we would use strata of HFE genotypes to distinguish effects within C282Y/wild-type and C282Y/H63D subjects. However, despite the fact this is the largest study to date, numbers of cases were still insufficient for reliable statistical analyses. Therefore, we recategorized HFE genotypes into noncarriers (wild-type/wild-type), carriers of 1 "risk" allele (H63D/wild-type, C282Y/wild-type), and carriers of 2 risk alleles (H63D/H63D, C282Y/H63D, C282Y/C282Y).

All analyses were conducted with the use of SAS statistical software version 9.1 (SAS Institute, Cary, NC). Reported probability values are 2-sided, and $P<0.05$ was considered statistically significant. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Our search strategy in PubMed resulted in 58 articles. When the formulated criteria were applied to these articles, 13 eligible studies[8,10–12,14–22] were identified. We did not come across any articles in the reference lists that were not already selected by our PubMed search. In addition, data from 1 unpublished study were included.[23] All of the studies agreed to participate and, with the exception of 3,[14,16,17] were able to provide the individual patient data, making the total number of data sets included in the joint analyses 11.

The characteristics of the included studies and participants are listed in Table 1. Control C282Y genotype frequencies of most studies were in Hardy-Weinberg equilibrium, except for the UK study of Hetet et al[10] ($P=0.04$) and Waalen et al[18] ($P=0.03$). Hardy-Weinberg equilibrium of the data of Gunn et al[22] was marginally significant ($P=0.08$). Control H63D genotype frequencies of all studies were in Hardy-Weinberg equilibrium, although 3 were marginally significant (van der A et al[8] [$P=0.06$], Candore et al[11] [$P=0.08$], and Waalen et al[18] [$P=0.06$]). The reported frequencies of the 282Y allele ranged from 0.0% in the south of Italy to 10.7% (95% CI, 8.8% to 12.6%) in North Glasgow, Scotland, showing a distinct north-south gradient across Europe. The allelic frequency of the H63D mutation varied much less, showing the lowest frequency again in the south of Italy (12.5%; 95% CI, 9.3% to 15.7%) and in Denmark (12.5%; 95% CI, 12.0% to 13.0%) and the highest in France (17.5%; 95% CI, 13.3% to 22.2%).

Table 2 shows the population characteristics of the IPD study. Data from 53 880 subjects were compiled in the central

Table 2. Population Characteristics of the IPD Study

Characteristics	Controls	CHD Cases	AMI Cases
No. of individuals	43 366	10 514	5724
Women, n (%)	22 830 (53)	3337 (32)	1356 (24)
Age, mean±SD, y	56 ±14	63 ±11	62 ±11
Current smoking, n (%)*	8701 (20)	2951 (30)	2123 (38)
Hypertension, n (%)*	11 758 (27)	4496 (46)	2508 (46)
Hypercholesterolemia, n (%)*	23 850 (55)	6903 (70)	3993 (72)
Type 2 diabetes, n (%)*	1926 (5)	1102 (12)	635 (13)
Frequency 282Y allele†	6.3 (6.1–6.4)	6.6 (6.3–7.0)	6.9 (6.5–7.4)
Frequency 63D allele†	14.3 (14.1–14.5)	14.8 (14.3–15.3)	14.8 (14.1–15.4)
HFE genotype, n (%)	43 366 (100)	10 514 (100)	5724 (100)
Wild-type/wild-type	27 361 (63)	6501 (62)	3520 (62)
H63D/wild-type	9835 (23)	2419 (23)	1313 (23)
H63D/H63D	920 (2)	245 (2)	129 (2)
C282Y/wild-type	4313 (10)	1100 (10)	608 (11)
C282Y/H63D	744 (2)	203 (2)	121 (2)
C282Y/C282Y	193 (<1)	46 (<1)	33 (<1)

*Percentages are based on the number of subjects with available data on this factor.
†Percentages and 95% CIs.

database, based on 43 366 controls and 10 514 CHD cases (including n=5724 AMI cases). Cases were more frequently male, were on average slightly older, and showed a higher prevalence of classic cardiovascular risk factors.

In Table 3, pooled ORs for the association between HFE genotypes and cardiovascular disease are presented. Estimates are shown for AMI and CHD risk and for combined HFE genotypes, carriers of the 282Y allele, and carriers of the 63D allele (see Figures I to IV in the online-only Data Supplement). Minimally adjusted models demonstrated a small increased, although not statistically significant, risk of AMI in compound heterozygous subjects compared with wild-type subjects (OR=1.10; 95% CI, 0.88 to 1.38). The OR increased slightly when we further adjusted for age, sex, and classic cardiovascular risk factors (OR=1.15; 95% CI, 0.88 to 1.50). When 282Y and 63D alleles were examined separately, carriers of the 282Y allele were not at increased risk for AMI compared with noncarriers (OR=0.95; 95% CI, 0.86 to 1.06), but for 63D carriers a small increased risk was seen (OR=1.08; 95% CI, 1.00 to 1.17) that approached statistical significance (P=0.07). Similar results were observed for CHD. Inclusion of a cross-product term in the models showed no evidence of interaction on a multiplicative scale between the C282Y and H63D alleles. Sensitivity analyses, excluding each study one at a time, were in general agreement with the overall results.

The results of the analysis of risk modification by HFE genotypes of the relation between classic risk factors and cardiovascular events, overall and stratified by carriers of varying numbers of risk alleles, are summarized in Table 4. We observed no evidence for effect modification of the relation between hypertension, smoking, diabetes, hypercholesterolemia, gender, age, and AMI by HFE genotypes. However, interaction between HFE genotypes (classified as carriers of risk alleles) and hypertension for CHD risk approached statistical significance (P=0.05). The effect was mainly ascribed to the study of Waalen et al[18] (P<0.005). Hypertension was associated with an increased risk of CHD, both in the presence and in the absence of HFE mutations. The association, however, was attenuated in carriers of 2 HFE risk alleles (OR=1.22; 95% CI, 0.93 to 1.59) compared with noncarriers (OR=1.71; 95% CI, 1.59 to 1.83).

Discussion

Our present findings do not provide evidence for a material role of HFE genotypes in cardiovascular disease risk. Despite a large sample size, none of the HFE genotypes showed a significant increase in risk compared with wild-types. Carriage of the H63D mutation was, however, related to a small but statistically significant increased risk of CHD (OR=1.08; 95% CI, 1.02 to 1.15) after adjustment for potential confounders. However, because we tested several hypotheses, findings with probability values near the nominally significant 0.05 level should be considered suggestive only.

We have examined the association between combined HFE genotypes and CHD risk in a meta-analysis based on individual patient data of 53 880 subjects from 11 different studies. This enabled us to distinguish the effects of single heterozygotes from compound heterozygotes. In our opinion, this is an important distinction that needs to be made in view of the evidence that significant differences in iron indices exist between the 2 groups.[24,25] Pooling of individual data of the separate studies into a meta-analysis markedly increases statistical power, not only for such analyses but also to explore the possibility that the relation between cardiovascular risk factors and CHD risk depends on HFE genotypes. Although this IPD meta-analysis is based on a large number

Table 3. Pooled ORs (95% CI) of the Association Between HFE Genotypes and Cardiovascular Disease

	No. (%) of Cases	Minimum Detectable Risk*	Model I†	Model I With Exclusion†‡	Model II§	Model III‖#
AMI						
Wild-type/wild-type	3520 (62)	…	1.0	1.0	1.0	1.0
H63D/wild-type	1313 (23)	1.10	1.04 (0.97–1.13)	1.07 (0.99–1.16)	1.03 (0.96–1.12)	1.06 (0.97–1.16)
H63D/H63D	129 (2)	1.30	1.14 (0.92–1.42)	1.13 (0.90–1.42)	1.16 (0.93–1.44)	1.12 (0.88–1.42)
C282Y/wild-type	608 (11)	1.14	1.02 (0.92–1.13)	0.99 (0.89–1.11)	1.00 (0.90–1.11)	0.94 (0.84–1.07)
C282Y/H63D	121 (2)	1.30	1.10 (0.88–1.38)	1.10 (0.87–1.41)	1.09 (0.86–1.38)	1.15 (0.88–1.50)
C282Y/C282Y	33 (<1)	1.63	1.01 (0.65–1.57)	0.97 (0.58–1.64)	1.12 (0.71–1.77)	0.94 (0.50–1.76)
282Y allele (−)	4962 (87)	…	1.0	1.0	1.0	1.0
282Y allele (+)	762 (13)	1.13	1.02 (0.93–1.12)	0.99 (0.89–1.09)	1.00 (0.91–1.11)	0.95 (0.86–1.06)
63D allele (−)	4161 (73)	…	1.0	1.0	1.0	1.0
63D allele (+)	1563 (27)	1.09	1.05 (0.98–1.13)	1.08 (1.00–1.16)	1.05 (0.97–1.12)	1.08 (1.00–1.17)
Total	5724 (100)	…	…	…	…	…
CHD						
Wild-type/wild-type	6501 (62)	…	1.0	1.0	1.0	1.0
H63D/wild-type	2419 (23)	1.07	1.06 (1.00–1.12)	1.07 (1.01–1.14)	1.06 (1.00–1.12)	1.07 (1.00–1.14)
H63D/H63D	245 (2)	1.23	1.19 (1.02–1.40)	1.19 (1.01–1.40)	1.19 (1.01–1.40)	1.16 (0.97–1.38)
C282Y/wild-type	1100 (10)	1.10	1.03 (0.95–1.11)	1.02 (0.94–1.10)	1.02 (0.94–1.10)	0.98 (0.90–1.07)
C282Y/H63D	203 (2)	1.23	1.08 (0.91–1.29)	1.08 (0.90–1.30)	1.09 (0.91–1.30)	1.12 (0.92–1.37)
C282Y/C282Y	46 (<1)	1.48	0.84 (0.59–1.21)	0.79 (0.52–1.19)	0.92 (0.63–1.33)	0.78 (0.49–1.26)
282Y allele (−)	9165 (87)	…	1.0	1.0	1.0	1.0
282Y allele (+)	1349 (13)	1.10	1.01 (0.94–1.09)	0.99 (0.92–1.07)	1.01 (0.94–1.08)	0.97 (0.90–1.05)
63D allele (−)	7647 (73)	…	1.0	1.0	1.0	1.0
63D allele (+)	2867 (27)	1.07	1.07 (1.01–1.12)	1.08 (1.03–1.14)	1.07 (1.01–1.13)	1.08 (1.02–1.15)
Total	10 514 (100)	…	…	…	…	…

*The OR that can be detected at 80% power at 2-sided $P \leq 0.05$.

†Adjusted for study (AMI analyses based on n=49 090 subjects; CHD analyses based on n=53 880 subjects).

‡Excluding the studies by Candore et al[11] and Hetet et al[10] (AMI analyses based on n=47 059 subjects; CHD analyses based on n=51 849 subjects).

§Adjusted for study, age, and gender (AMI analyses based on n=49 068 subjects; CHD analyses based on n=53 858 subjects).

‖Adjusted for study, age, gender, current smoking, hypertension, hypercholesterolemia, type 2 diabetes (AMI analyses based on n=46 597; CHD analyses based on n=50 795 subjects).

#Assuming additive allele effects on a logistic scale (coding each genotype as an ordinal variable (0, 1, 2)) the results using model III for CHD were OR=0.98 (0.91–1.06) for C282Y and OR=1.08 (1.02–1.13) for H63D. For AMI the results were OR=0.97 (0.87–1.07) for C282Y and OR=1.07 (0.99–1.15) for H63D.

of subjects, it may still have insufficient power to detect weak genetic effects for certain HFE genotypes.

Previous studies showing an association between excess iron and CHD risk have reported a stronger effect in the presence of other cardiovascular risk factors such as smoking, hypertension, or high levels of low-density lipoprotein.[2,26–28] These synergistic associations may complicate the ability to detect a possible association between iron and CHD. It might well be possible that slightly elevated iron levels are only deleterious in conjunction with increased oxidative stress caused by additional cardiovascular risk factors. In this meta-analysis, we observed no clear evidence for such effect modification (showing a different effect of HFE genotypes on CHD risk in individuals within different risk factor strata, or, conversely, showing a different effect of classic risk factors on CHD risk in persons with different HFE genotypes). Unfortunately, even with a large sample size like ours, our study was still limited by insufficient power to adequately evaluate such interactions with individual HFE genotypes. We were therefore compelled to lump together certain HFE genotypes, which in a way ignores our original hypothesis that the effect for compound heterozygous individuals might be higher than for single heterozygous individuals. We cannot exclude the possibility of an interaction between carriage of HFE mutations and hypertension ($P=0.05$). In our data, hypertension seems to be a less important risk factor for CHD when more risk alleles are present. This finding is in contrast with the hypothesis that the effects of classic risk factors and excess iron are synergistic.

To avoid bias, we attempted to include all available studies in our meta-analysis. Unfortunately, 3 studies had to be withdrawn from our study because the authors were unable to retrieve their data.[14,16,17] The published results of these studies, however, are largely consistent with the findings of our meta-analysis, suggesting that their inclusion would not have changed our conclusions.

From a methodological point of view, one can criticize the fact that we ignored different study designs in our statistical analysis. However, we generally obtained similar results

Table 4. Pooled ORs (95% CI) of the Association Between Conventional Cardiovascular Risk Factors and Cardiovascular Disease, Overall and Stratified by HFE Genotypes

Factor	Overall*	Noncarriers†	Carriers of 1 Risk Allele†	Carriers of 2 Risk Alleles†	*P* for Interaction
AMI					
Hypertension	1.55 (1.43–1.67)	1.54 (1.40–1.70)	1.59 (1.39–1.81)	1.24 (0.85–1.80)	0.53
Smoking	1.37 (1.26–1.49)	1.34 (1.20–1.49)	1.39 (1.20–1.61)	1.65 (1.10–2.47)	0.64
Diabetes	2.42 (2.15–2.73)	2.50 (2.15–2.92)	2.32 (1.89–2.85)	2.10 (1.21–3.63)	0.77
Hypercholes.	4.66 (4.21–5.16)	4.72 (4.16–5.36)	4.53 (3.79–5.42)	4.59 (2.80–7.52)	0.71
Male gender	3.77 (3.47–4.10)	3.72 (3.35–4.14)	3.88 (3.34–4.51)	3.90 (2.62–5.79)	0.83
Age >58 y‡	2.09 (1.93–2.25)	2.17 (1.96–2.39)	2.00 (1.75–2.29)	1.69 (1.18–2.44)	0.39
CHD					
Hypertension	1.66 (1.57–1.75)	1.71 (1.59–1.83)	1.63 (1.48–1.79)	1.22 (0.93–1.59)	0.05
Smoking	1.16 (1.09–1.24)	1.17 (1.07–1.27)	1.12 (1.00–1.26)	1.38 (1.01–1.88)	0.52
Diabetes	2.07 (1.89–2.26)	2.08 (1.85–2.34)	2.13 (1.83–2.49)	1.53 (0.99–2.36)	0.30
Hypercholes.	3.14 (2.93–3.38)	3.29 (3.00–3.60)	2.92 (2.58–3.31)	2.96 (2.11–4.16)	0.31
Male gender	2.33 (2.20–2.46)	2.35 (2.19–2.53)	2.28 (2.06–2.51)	2.40 (1.85–3.13)	0.99
Age >58 y‡	2.07 (1.96–2.19)	2.14 (1.99–2.30)	1.94 (1.76–2.14)	2.13 (1.63–2.78)	0.21

*Model includes carriage of HFE mutations, study, age (continuous), gender, current smoking, hypertension, hypercholesterolemia, and type 2 diabetes, except for ‡, in which the model includes age (dichotomous).

†Model includes study, age (continuous), gender, current smoking, hypertension, hypercholesterolemia, and type 2 diabetes, except for ‡, in which the model includes age (dichotomous).

when we selected only the case-control and cross-sectional studies as when we included all designs (data not shown). The reduction in number of subjects only slightly affected the estimates for genotypes with the smallest prevalence. Sensitivity analyses, excluding 1 study at a time, were in general agreement with the overall results.

The frequency of the studied polymorphisms varies substantially across Europe, whereas the frequency of the outcomes also varies across the European population, but in a direction opposite to the allele frequency changes. This could result in a population substructure masking an association. We stratified for study in our analyses to address this issue. However, substructure within studies could also mask associations. This would be of greatest concern for populations likely to be of mixed ancestry such as the studies from the United States[18] and Australia,[19] consisting of subjects likely to have ancestry from across Europe. We cannot exclude the possibility that cryptic population structure is masking an association.

Although not all published studies could be included, our findings are comparable with 2 previous meta-analyses based on aggregated data,[8,29] and similar conclusions are drawn. For 63D carriers, the slightly higher risk (OR=1.08; 95% CI, 1.02 to 1.15) that was observed in the current meta-analysis could be in accordance with the effect estimated from the aggregated data (OR=1.03; 95% CI, 0.96 to 1.11) because the CIs around the estimates still overlap.

Last year, results were published of the first large prospective randomized trial testing the hypothesis that a reduction in body iron stores through phlebotomy has an effect on all-cause mortality and death plus nonfatal myocardial infarction and stroke in patients with peripheral arterial disease.[30] No statistically significant difference was observed between the iron-reduction group and the control group for any end point, although ferritin levels decreased significantly by 35%. However, this study had only 68% power to detect a rather large reduction in mortality (30%).

Previous studies found small differences in ferritin levels between compound heterozygous and wild-type individuals, which ranged between 19 and 80 μg/L.[25,31,32] However, subjects with heterozygosity for C282Y and H63D typically have normal iron stores.[31–33] In a large trial, only 20% of male heterozygotes and 8% of female heterozygotes had serum ferritin levels that exceeded the 95th percentile values.[34] It is possible that, in addition to environmental effects on iron metabolism, other genetic factors affect the severity of iron overload. On the basis of our results, we are unable to reject or support the original iron hypothesis for atherosclerosis proposed by Sullivan, but further studies should be illuminating.

In plasma of C282Y heterozygotes, a significantly increased concentration of non–transferrin-bound iron has been found.[35] In vitro studies have demonstrated that this prooxidative iron species is able to stimulate expression of adhesion proteins on endothelial cells, which can be inhibited with iron chelators.[36,37] However, an association between non–transferrin-bound iron levels and CHD could not be demonstrated previously,[38] nor did we find associations between C282Y heterozygosity and AMI or CHD risk in this study. Although experimental studies identified iron as one of the numerous modifiers of vascular function, the exact pathophysiological impact on early stages of atherosclerosis and on cardiovascular outcomes remains uncertain. Moreover, the exact role of the HFE gene in cardiovascular disease, either causal, for example, via new biomarkers such as non–transferrin-bound iron or other forms of labile iron, or indirect, via linkage or modification of nearby genes, needs to be elucidated further.

In conclusion, the results of our large IPD meta-analysis do not support the view that HFE gene mutations are associated

with an increased risk of CHD or AMI. However, the power of our study does not exclude weak genetic effects on CHD (OR <1.23) or AMI (OR <1.30) by the C282Y/H63D genotype.

Disclosures.

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CLINICAL PERSPECTIVE

In the early eighties Sullivan proposed that an increase in body iron levels and subsequent oxidative stress may play a role in the occurrence of cardiovascular disease. Up until 1996 blood markers of iron status and dietary iron intake were mostly studied as indicators of iron exposure, but from that year on new opportunities were opened due to the discovery of the hemochromatosis related gene HFE. The two most important mutations in the HFE gene, 282C→Y and 63H→D, have both been associated with varying degrees of iron overload. However, their role as a risk factor for cardiovascular disease remains subject to debate. In this Individual Patient Data (IPD) meta-analysis based on more than 10,000 documented cases of CHD (of which almost 6,000 AMI cases) from eleven observational studies, we found no evidence for a material role of HFE genotypes in cardiovascular disease risk. However, the power of our study does not exclude weak genetic effects on CHD (odds ratio <1.23) or AMI (odds ratio <1.30) by the C282Y/H63D genotype.