

Increased Risk of Acute Myocardial Infarction in Carriers of the Hemochromatosis Gene Cys282Tyr Mutation

A Prospective Cohort Study in Men in Eastern Finland

Tomi-Pekka Tuomainen, MD; Kimmo Kontula, MD, PhD; Kristiina Nyysönen, PhD; Timo A. Lakka, MD, PhD; Tiina Heliö, MD, PhD; Jukka T. Salonen, MD, PhD, MScPH

Background—Homozygosity for a relatively common Cys282Tyr mutation of the human hemochromatosis-associated (*HFE*) gene was recently found to account for most cases of hereditary hemochromatosis. Because excess iron has been postulated to enhance risk of vascular disease, we studied whether occurrence of this mutation was associated with increased risk of first acute myocardial infarction in healthy middle-aged men in a prospective cohort study.

Methods and Results—Study subjects were the 1150 participants in the population-based Kuopio Ischemic Heart Disease Risk Factor Study (KIHD), aged 42, 48, 54, or 60 years at baseline, who had no coronary heart disease at baseline and for whom a DNA sample was available. Information about myocardial infarctions was collected prospectively by use of FINMONICA (FINnish MONItoring of trends and determinants in CARDiovascular disease study) and hospital data. Events were classified by MONICA (MONItoring of trends and determinants in CARDiovascular disease study) diagnostic criteria. The *HFE* Cys282Tyr mutation was assayed by a solid-phase minisequencing technique. One subject was homozygous and 76 individuals were heterozygous for the *HFE* Cys282Tyr mutation (6.7%). During a mean follow-up of 9 years, 8 (10.4%) of 77 carriers and 60 (5.6%) of 1073 noncarriers experienced an acute myocardial infarction. In a Cox proportional hazards model allowing for the other strongest risk factors, the carriers had a 2.3-fold (95% CI 1.1 to 4.8; $P=0.03$) risk of acute myocardial infarction compared with noncarriers.

Conclusions—Male carriers of the common hemochromatosis gene mutation are at 2-fold risk for first acute myocardial infarction compared with noncarriers. (*Circulation*. 1999;100:1274-1279.)

Key Words: epidemiology ■ hemochromatosis ■ iron ■ myocardial infarction

Hereditary hemochromatosis (HH), resulting in excess iron accumulation in the body and a variety of ensuing clinical manifestations, is one of the most common inherited metabolic disorders.¹ The mode of inheritance is autosomal recessive, and the estimated frequency of homozygosity may approach 5/1000 and the frequency of heterozygosity 1/10 in populations of northern European origin.^{1,2}

See p 1260

Identification of asymptomatic carriers of the trait has been complicated. Attempts to this end include screening of the population with ≥ 1 biochemical marker of iron status, followed by liver biopsy and evaluation of hepatic iron index, or by HLA-haplotyping of the first-degree relatives of symptomatic subjects to demonstrate HLA identity.¹⁻³ These approaches are not feasible for population studies, because unaffected homozygotes and the majority of heterozygous carriers would be missed. A more sensitive approach is to

monitor the disease-associated (or preferably the disease-causing) gene itself.

Recently, Feder and coworkers⁴ found that a specific mutation (Cys282Tyr) of a novel hemochromatosis-associated gene, initially called HLA-H but subsequently renamed *HFE*, was present in a homozygous form in 85% of their HH patients of northern European origin. It is now thought that this is the main mutation that causes hemochromatosis.

Because we previously demonstrated that increased body iron stores are an independent risk factor for acute myocardial infarction (AMI),^{5,6} we studied whether male carriers of the *HFE* Cys282Tyr mutation are at an increased risk of first AMI.

Methods

Subjects

The study subjects were participants of the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD),⁷ a prospective population study

Received December 29, 1998; revision received June 8, 1999; accepted June 17, 1999.

From the Research Institute of Public Health, University of Kuopio, Kuopio, Finland (T.-P.T., K.N., T.A.L., J.T.S.); the Department of Community Health and General Practice, University of Kuopio, Kuopio, Finland (T.-P.T., J.T.S.); and the Department of Medicine, University of Helsinki, Helsinki, Finland (K.K., T.H.).

Correspondence to Professor Jukka T. Salonen, Research Institute of Public Health, University of Kuopio, Harjulantie 1 B, 70210 Kuopio, Finland (or PO Box 1627, 70211 Kuopio, Finland). E-mail jukka.salonen@uku.fi

© 1999 American Heart Association, Inc.

Circulation is available at <http://www.circulationaha.org>

to investigate previously unestablished risk factors for AMI, atherosclerosis, and other related outcomes. The KIH D study sample is a random third of men from eastern Finland who were aged 42, 48, 54, or 60 years at study entry. At baseline, during 1984 to 1989, 2682 men (82.9% of the eligible 3235) were examined. The study protocol was approved by the Research Ethics Committee of the University of Kuopio, and all participants gave written informed consent.

To study the role of hemochromatosis in the first manifestations of coronary heart disease (CHD), men with history of CHD at baseline were excluded. CHD history was defined as either a history of AMI or angina pectoris, positive angina pectoris on effort according to the Rose questionnaire, or use of nitroglycerin tablets at least weekly. A DNA sample was available for 1150 CHD-free men.

HFE Cys282Tyr Genotyping

DNA was extracted from 10 mL of EDTA-anticoagulated venous blood by standard salting-out or phenol-chloroform assays. The G-to-A transition at nucleotide 845 of the *HFE* cDNA, resulting in a substitution of tyrosine for cysteine at codon 282, was assayed by a solid-phase minisequencing technique.⁸ In short, a DNA fragment containing the mutation point was first amplified by polymerase chain reaction (PCR) with the primers 5'-CTC AGG CAC TCC TCT CAA CC-3' (biotinylated at its 5'-end) and 5'-TGG CAA GGG TAA ACA GAT CC-3'. The amplified 5'-biotinylated PCR products were captured in streptavidin-coated microtitration wells, washed, and denatured. The variable nucleotide (G/A) in the immobilized DNA was assayed with a primer extension reaction directed by a detection step primer (5'-GGA AGA GCA GAG ATA TAC GT-3') annealing to the DNA immediately upstream of the site of variation. In this elongation reaction, a single tritiated nucleoside triphosphate is incorporated into the DNA. After the wells were washed, the elongated primer was released by addition of 50 mmol/L NaOH and counted for radioactivity. All samples positive for the presence of the Cys282Tyr mutation were reassayed by PCR amplification followed by *RsaI* restriction-enzyme analysis and polyacrylamide gel electrophoresis, and identical results were obtained. Assays for the *HFE* Cys282Tyr mutation were always performed blindly in relation to other data of the respective individual.

Measurement of Other Risk Factors

The KIH D examination protocol and collection of samples have been described previously.^{5,7} The measurement of serum VLDL, LDL, and HDL cholesterol concentrations,⁵ serum HDL₂ and HDL₃ sub-fractions,⁵ serum total cholesterol and triglycerides,⁵ and serum apolipoprotein B and A-I⁵ has been described previously. Serum apolipoprotein(a) concentrations were determined with a radioimmunoassay [Mercodia Apo(a) RIA]. The measurement of blood leukocyte count,⁶ serum γ -glutamyl transferase activity,⁶ plasma fibrinogen,⁹ plasma vitamin C¹⁰ and E (lipid standardized),¹¹ serum ferritin,⁵ serum copper,¹⁰ hair mercury content,⁹ and 24-hour urinary nicotine metabolites¹² has also been described previously.

Details about the measurement of blood pressure⁵ and maximal oxygen uptake,⁶ as well as criteria for exercise-induced ischemia,⁵ are also provided in previous reports. Waist-to-hip circumference ratio (WHR) was calculated as the circumference of the waist (in centimeters) divided by the circumference of the hip (in centimeters), and body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Assessment of medical history,⁵ smoking,⁵ alcohol consumption,⁶ socioeconomic score,¹¹ energy expenditure of conditioning leisure-time physical activity,⁶ and dietary intake of nutrients⁶ was performed as described previously.

Collection and Coding of AMI Data

The province of Kuopio participated in the multinational MONICA (MONItoring of Trends and Determinants in Cardiovascular Disease) project,¹³ in which detailed diagnostic information of suspected AMI during 1982 to 1992 was collected prospectively by the FINMONICA (Finnish MONICA) investigator group. Data on AMI between January 1993 and December 1996 were obtained by record

linkage from the national computerized hospital discharge registry. Diagnostic information was collected from hospitals and classified by identical diagnostic criteria. The follow-up time to AMI, death, or December 31, 1996, varied between 30 days and 12.8 years, with a mean of 9.1 years. In the case of multiple events for the same subject, only the first event was considered a follow-up end point. Thirty-three of the events were classified as definite AMI, 24 as possible AMI, and 11 as prolonged chest pain according to MONICA criteria.

Statistical Methods

Differences in risk factors between subjects who experienced an AMI during follow-up and those who had no AMI were tested for statistical significance with the Student *t* test, allowing for unequal variances.

Risk factor-adjusted relative risks of AMI were estimated as relative hazards by the Cox proportional hazards model. We examined the fit of the model by analyzing changes in the proportionality of hazards with time and risk factors. The genotype at codon 282 of the *HFE* gene was dichotomized as Cys/Cys=0 and Cys/Tyr or Tyr/Tyr=1. To avoid loss of information, covariates were entered as continuous variables.

Covariates for the model were selected 1 at a time by Cox models, with age and examination year and month entered simultaneously. Of a large number of previously known or predictive risk factors tested, a set of 13 risk factors was used in further analyses as covariates (Table 2). Other factors tested that were not significant and therefore were not entered as covariates were serum concentrations of apolipoprotein A-I (mg/L); HDL₃ cholesterol (mmol/L); triglycerides (mmol/L); apolipoprotein(a) (mg/L); γ -glutamyl transferase (U/L); copper (mg/L) and lipid-standardized vitamin E; blood leukocyte count (10⁹/L); alcohol consumption (g/wk); hair mercury content (μ g/g); use of antihypertensive medication for hypertension (yes versus no); BMI (kg/m²); diastolic blood pressure (mm Hg); intensity (metabolic equivalents [METs]) and duration (h/wk) of conditioning leisure-time physical activity; daily intake of saturated, monounsaturated, and polyunsaturated fatty acids (g) and iron (mg); and number of hangovers per year.

Missing values in covariates were replaced by means. All tests of statistical significance were 2-sided. The statistical analyses were computed with SPSS statistical software.

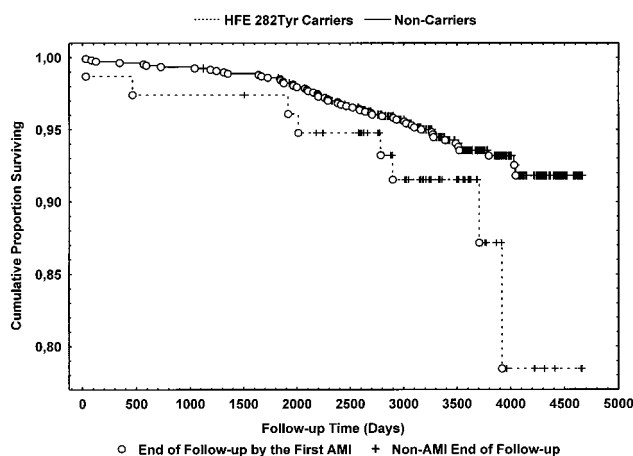
Results

Prevalence of the Tyr282 Allele in Its Relation to AMI

There were 1 homozygous and 76 heterozygous individuals for the *HFE* Tyr282 allele among the 1150 study subjects. Thus, the overall frequency of the Tyr282 allele was 3.4%, and the carrier frequency was 6.7%. Eight AMIs were registered in the 77 Tyr282 carriers (AMI incidence 10.4%) and 60 infarctions in the 1073 noncarriers (AMI incidence 5.6%) during the follow-up, totalling 68 events (Figure).

Risk Factor Characteristics

Baseline distributions of the strongest risk factors of AMI in subjects who did and did not experience an AMI during the 9 years of follow-up are shown in Table 1. There were no statistically significant differences in the 13 covariates between *HFE* Tyr282 carriers and noncarriers. The mean serum ferritin concentration for carriers and noncarriers of the mutation was 175 (SE 4.5) and 165 (SE 20.1) μ g/L, respectively. The proportion of subjects with serum ferritin >200 μ g/L was 7.3% for carriers and 6.5% for noncarriers of the mutation.



AMI survival of carriers and noncarriers of hemochromatosis gene *HFE* 282Tyr allele in 1150 eastern Finnish men.

AMI Incidence According to Presence of *HFE* Tyr282 Allele

The unadjusted relative risk of AMI for Tyr282 carriers was 1.95 (95% CI 0.93 to 4.08; $P=0.08$). The 13 strongest risk factors were entered in a forward stepwise Cox proportional-hazards model. The analysis selected *HFE* genotype and 6 other risk factors: serum apolipoprotein B concentration, 24-hour urinary excretion of nicotine metabolites, ischemic ECG finding in the baseline exercise test, age, WHR, and serum VLDL cholesterol concentration (P to enter 0.05) (Table 2, model 1). In this model, carriers of the Tyr282 allele had >2-fold increased risk of AMI (relative risk 2.29, 95% CI 1.09 to 4.83; $P=0.03$) compared with noncarriers. In a model in which all 13 risk factors were forced in, the relative risk for an AMI in carriers versus noncarriers was 2.21 (95% CI 1.05 to 4.67; $P=0.04$) (Table 2, model 2). This relative risk was similar in analysis based on definite and possible AMIs only.

The population attributable risk for the *HFE* Cys282Tyr mutation was 8.0% (95% CI 0.6 to 20.4%) for the stepwise

analysis and 7.5% (95% CI 0.3 to 19.7%) when all 13 risk factors were forced into the model. According to this analysis, $\approx 8\%$ of the first AMIs within the study population were explained by this mutation.

The interaction between body iron stores and Tyr282 allele carrier status on the risk of AMI was studied separately in *HFE* Tyr282 carriers and noncarriers in a model including serum ferritin concentration, age, and examination year and month. In carriers, a rise of every 100 μg of ferritin per liter increased the risk of AMI by 52% (95% CI 11% to 107%; $P=0.009$). Serum ferritin concentration was not predictive of AMI among noncarriers of the *HFE* Cys282Tyr mutation (risk increase of 0.6% for every 100 μg of serum ferritin per liter; $P=0.95$).

To study the synergism between *HFE* 282Tyr allele carrier status and the major coronary risk factors with regard to the risk of AMI, the interactions between *HFE* 282Tyr allele carrier status and systolic blood pressure, serum LDL cholesterol, and smoking were explored. The relative risk of AMI associated with the mutation was 2.33 (95% CI 0.99 to 5.50; $P=0.05$) in men with high serum LDL cholesterol concentration (divided at median) and 1.44 (95% CI 0.33 to 6.24; $P=0.62$), in men with low serum LDL cholesterol concentration. The respective relative risk of AMI was 2.49 (95% CI 1.05 to 5.90; $P=0.04$) in men with high systolic blood pressure (divided at median) and 1.25 (95% CI 0.29 to 5.30; $P=0.74$) in men with low systolic blood pressure. The relative risk of AMI associated with the *HFE* mutation was also higher in smokers than in nonsmokers (2.16 versus 1.69), although this was not statistically significant in either group. We adjusted for age and examination year and month in these analyses. The relative risks did not differ statistically significantly between subgroups.

Discussion

Our study suggests an increased risk of AMI in men who are carriers of the *HFE* Cys282Tyr mutation. This *HFE* gene

TABLE 1. Baseline Distributions of the Strongest Risk Factors for AMI in Subjects Who Did (n=68) and Did Not (n=1082) Experience an AMI During the 9 Years of Follow-Up

Characteristic	AMI		Non-AMI		<i>P</i> for Difference
	Mean \pm SD	Range	Mean \pm SD	Range	
Serum apolipoprotein B, g/L	1.2 \pm 0.2	0.7–1.7	1.0 \pm 0.2	0.2–1.9	<0.001
Serum HDL ₂ cholesterol, mmol/L*	0.8 \pm 0.2	0.3–1.3	0.9 \pm 0.3	0.3–2.3	<0.001
Maximal oxygen uptake, L/min	2.3 \pm 0.5	1.2–3.8	2.6 \pm 0.6	0.7–5.5	<0.001
Ischemic ECG finding in exercise test at baseline, number of subjects (%)	24 (35%)		162 (15%)		<0.001
Age, y	54.4 \pm 4.8	42.1–61.1	51.9 \pm 5.9	42.0–61.2	0.001
WHR, cm/cm	1.0 \pm 0.1	0.8–1.1	0.9 \pm 0.1	0.8–1.5	0.003
Plasma fibrinogen, g/L	3.1 \pm 0.6	2.2–6.7	3.0 \pm 0.5	1.7–6.3	0.009
Socioeconomic status, score†	9.0 \pm 3.9	1.0–17.0	7.4 \pm 4.1	0.0–17.0	0.01
Plasma vitamin C, mg/L	7.4 \pm 4.4	0.5–17.5	8.9 \pm 4.0	0.3–24.2	0.02
Urinary excretion of nicotine metabolites, mg/d	7.5 \pm 10.2	0.0–51.1	5.1 \pm 6.7	0.0–59.4	0.04
Systolic blood pressure, mm Hg	138 \pm 17	104–195	133 \pm 16	99–201	0.07
Serum VLDL cholesterol, mmol/L	0.8 \pm 1.5	0.0–12.8	0.6 \pm 0.4	0.0–3.6	0.67

Distributions of examination year and month are not shown.

*To convert values for cholesterol to milligrams per deciliter, divide by 0.02586.

†High score denotes low socioeconomic status.

TABLE 2. Adjusted Relative Hazards for First AMI in a Population-Based Prospective Sample of 1150 Eastern Finnish Men With No History of CHD in 9 Years of Follow-Up

Risk Factor	Model 1*		Model 2†	
	Relative Hazard (95% CI)	P	Relative Hazard (95% CI)	P
HFE 282 Tyr, yes vs no	2.29 (1.09–4.83)	0.03	2.21 (1.05–4.67)	0.04
Ischemic ECG finding in exercise test at baseline examinations, yes vs no	2.31 (1.39–3.85)	0.001	2.16 (1.27–3.68)	0.005
24-Hour urinary excretion of nicotine metabolites, mg/d	1.04 (1.01–1.06)	0.001	1.03 (1.01–1.06)	0.007
Serum apolipoprotein B, 100 mg/L	1.18 (1.08–1.31)	0.001	1.16 (1.03–1.30)	0.01
Age, y	1.08 (1.03–1.13)	0.003	1.06 (1.00–1.12)	0.04
WHR×10, cm/cm	1.63 (1.11–2.38)	0.01	1.45 (0.94–2.25)	0.09
Serum VLDL cholesterol, mmol/L‡	1.41 (1.05–1.91)	0.02	1.35 (0.95–1.91)	0.10
Systolic blood pressure, mm Hg	...		1.01 (1.00–1.03)	0.18
Serum HDL ₂ cholesterol, mmol/L	...		0.58 (0.19–1.79)	0.34
Plasma vitamin C concentration, mg/L	...		0.97 (0.91–1.03)	0.35
Socioeconomic status, score§	...		1.03 (0.96–1.10)	0.44
Maximal oxygen uptake, L/min	...		0.83 (0.50–1.39)	0.49
Plasma fibrinogen, mg/L	...		1.13 (0.72–1.78)	0.60

*Forward stepwise Cox proportional hazards model, *P* for entry 0.05.

†Cox proportional hazards model with all 12 risk factors shown and examination year and month forced in.

‡To convert values for cholesterol to milligrams per deciliter, divide by 0.02586.

§High score denotes low socioeconomic status.

mutation is very strongly associated with the risk of human HH,⁴ and there is increasing evidence to support its role as the disease-causing mutation in most cases of this disease.^{14,15} The carrier frequency of the Tyr282 allele in most European populations is relatively high, ranging from 1% to 10%.¹⁶ In Finland, a population-based phenotype study suggested an HH prevalence of 50/100 000,¹⁷ which would give an estimated carrier frequency of 4% to 5%. Our genotype-based estimate (6.7%) is somewhat higher.

The Cys282Tyr mutation abolishes 1 of the 4 cysteine residues of the wild-type protein, destroying the β_2 -microglobulin binding capacity of HFE,¹⁸ and HFE knockout mice develop hepatic iron overload even on a standard diet.¹⁹ Furthermore, the intact HFE protein forms a stable complex with the transferrin receptor, thus lowering its affinity for transferrin,²⁰ whereas the mutant protein both lacks this activity²¹ and localizes mainly intracellularly.²¹ Taken together, there is ample evidence that the Cys282Tyr-mutated HFE protein contributes to iron accumulation in HH. Although another His63Asp variant of the HFE gene may account for a small fraction of HH inheritance,⁴ its exact significance as a potential HH gene remains open.

We have previously demonstrated an association between increased body iron and excess risk of AMI in middle-aged men in the prospective KIH population study.^{5,6} In the same population, we also observed an association between voluntary blood donations and reduced risk of AMI.^{22,23} According to our hypothesis, excess body iron contributes to the risk of AMI through enhanced free radical stress.^{24,25} Whether this iron-mediated oxidative stress acts more in the circulation, in the vessel wall, or in the myocardium has not yet been resolved. Iron has been found in excess in human atherosclerosis,^{26,27} where it coexists with ceroid.²⁸ In addition, iron

chelators have been shown to reduce ischemia-reperfusion injury and contractile dysfunction in animal models.^{29,30} These data suggest that in addition to promoting atherogenic lipid peroxidation, catalytic iron could have direct vulnerability-increasing effects in atherosclerosis and that it may promote the expansion of ischemic injury when it is liberated in damaged myocytes.

The evidence from prospective human population studies is more inconsistent. Increased estimated body iron stores have been associated with increased risk of CHD death or AMI in some but not all studies.^{24,25}

Two previous studies^{31,32} have assessed the potential association between the HFE gene polymorphisms and CHD, with negative results. In both of these studies, the cases were selected on the basis of their already-established atherosclerotic disease, and no adjustments for any confounding factors were made. Furthermore, 1 of the studies included only coronary disease patients, with no control group.³¹ The disagreement of these findings with our observation can also be explained by the survival bias in the cross-sectional studies.

On the basis of the present study, the hemochromatosis gene HFE Cys282Tyr substitution affects ≈ 67 of every 1000 Finns. It is common enough to have public health importance if heterozygosity is established as a risk factor in any severe disease. In the present study, the carrier status for this mutation explained as much as 8% of first AMIs within the study population.

Heterozygosity for HH has not previously been considered to signify clinical relevance, because iron accumulation in these patients is modest compared with homozygous individuals. However, individuals judged to be heterozygous for hemochromatosis do have higher body iron stores than

healthy controls.^{33,34} Knowledge regarding any risk-increasing impact of moderately increased iron stores would be especially important, because iron depletion by venesection is economical and effective when started early enough.^{1,35}

Myocardial infarction is not generally regarded as a likely complication of human HH. In our view, this association has not been studied in an optimal setting. If only clinically affected HH patients and their first-degree relatives are studied, the selection of cases will be biased. The bias comes from loss of a fraction of undiagnosed affected individuals as well those who have died, possible inclusion of some undiagnosed cases among control subjects, and inclusion of well-treated affected individuals among cases. The association is therefore best studied by direct screening for the causative gene mutation in a prospective population study. Although the present study was performed in a prospective population-based cohort, there is one shortcoming that should be emphasized. The effect of HH on CHD incidence can be expected to be most apparent in younger individuals.³⁶ Because we studied subjects who were >42 years old at baseline, it is possible that the observed prevalence rate of homozygosity and, perhaps to a lesser extent, of heterozygosity for the *HFE* Cys282Tyr mutation is an underestimate due to some self-selection and survival biases, avoidable only in still-younger age groups. These biases can also explain why the mutation carriers had only mildly elevated serum ferritin levels compared with noncarriers in our study. In addition, because our study was an association study and the major histocompatibility complex gene region where the *HFE* gene locates is a very gene-rich region, the increased risk of an AMI could also be modified by some other, non-iron-metabolism-related gene coinherited with the *HFE* mutation.

Different populations represent different gene pools, implying that gene-disease associations are expected to vary between populations. For this reason, our present finding that the *HFE* Cys282Tyr gene mutation is associated with an increased risk of AMI requires confirmation in other populations in which this mutation is likewise common. Our study also raises important questions about whether screening for HH at young ages might be justified in families at high risk for vascular disease and whether subsequent monitoring of iron status and iron-depleting treatment will eventually constitute new measures in the primary prevention of CHD.

Acknowledgments

This work was supported by the Academy of Finland (grants 41471, 1041086, and 2041022 to Dr Salonen and grant 35313 to Dr Kontula) and the National Heart, Lung, and Blood Institute (grant HL-44199 to Dr Kaplan). We are indebted to Terhi Nissinen, MSc, for preparation of DNA samples, to Tuula Soppela-Loponen for her assistance in *HFE* genotypings, and to the staff of the Research Institute of Public Health, University of Kuopio, for other data collection in the KIHD study.

References

1. Witte DL, Crosby WH, Edwards CQ, Fairbanks VF, Mitros FA. Practice Guideline Development Task Force of the College of American Pathologists: hereditary hemochromatosis. *Clin Chim Acta*. 1996;245:139–200.
2. Edwards CQ, Griffen LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP. Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. *N Engl J Med*. 1988;318:1355–1362.
3. Edwards CQ, Kushner JP. Screening for hemochromatosis. *N Engl J Med*. 1993;328:1616–1620.
4. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R Jr, Ellis MC, Fullan A, Hinton LM, Jones NL, Kimmel BE, Kronmal GS, Lauer P, Lee VK, Loeb DB, Mapa FA, McClelland E, Meyer NC, Mintier GA, Moeller N, Moore T, Morkang E, Prass CE, Drayna DT, Risch NJ, Bacon BR, Wolff RK. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet*. 1996;13:399–408.
5. Salonen JT, Nyyssönen K, Korpela H, Tuomilehto J, Seppänen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation*. 1992;86:803–811.
6. Tuomainen T-P, Punnonen K, Nyyssönen K, Salonen JT. Association between body iron stores and the risk of acute myocardial infarction in men. *Circulation*. 1998;97:1461–1466.
7. Salonen JT. Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study. *Ann Clin Res*. 1988;20:46–50.
8. Syvänen A-C, Aalto-Setälä K, Harju L, Kontula K, Söderlund H. A primer-guided nucleotide incorporation assay in the genotyping of apolipoprotein E. *Genomics*. 1990;8:684–692.
9. Salonen JT, Seppänen K, Nyyssönen K, Korpela H, Kauhanen J, Kantola M, Tuomilehto J, Esterbauer H, Tatzber F, Salonen R. Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in eastern Finnish men. *Circulation*. 1995;91:645–655.
10. Nyyssönen K, Parviainen MT, Salonen R, Tuomilehto J, Salonen JT. Vitamin C deficiency and risk of myocardial infarction: prospective population study of men from eastern Finland. *BMJ*. 1997;314:634–638.
11. Salonen JT, Nyyssönen K, Tuomainen T-P, Mäenpää PH, Korpela H, Kaplan GA, Lynch J, Helmrich SP, Salonen R. Increased risk of non-insulin dependent diabetes mellitus at low plasma vitamin E concentrations: a four year follow-up study in men. *BMJ*. 1995;311:1124–1127.
12. Puhakainen EVJ, Barlow RD, Salonen JT. An automated colorimetric assay for urine nicotine metabolites: a suitable alternative to cotinine assays for the assessment of smoking status. *Clin Chim Acta*. 1987;170:255–262.
13. Tuomilehto J, Kuulasmaa K. WHO MONICA Project: assessing CHD mortality and morbidity. *Int J Epidemiol*. 1989;18:S38–S45.
14. Mura C, Nousbaum J-B, Verger P, Moalic MT, Raguene O, Mercier AY, Ferec C. Phenotype-genotype correlation in hemochromatosis subjects. *Hum Genet*. 1997;101:271–276.
15. Adams PC, Chakrabarti S. Genotypic/phenotypic correlations in genetic hemochromatosis: evolution of diagnostic criteria. *Gastroenterology*. 1998;114:319–323.
16. Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJ. Global prevalence of putative hemochromatosis mutations. *J Med Genet*. 1997;34:275–278.
17. Karlsson M, Ikkala E, Reunanen A, Takkunen H, Vuori E, Mäkinen J. Prevalence of hemochromatosis in Finland. *Acta Med Scand*. 1988;224:385–390.
18. Feder JN, Tsuchihashi Z, Irrinki A, Lee VK, Mapa FA, Morikang E, Prass CE, Starnes SM, Wolff RK, Parkkila S, Sly WS, Schatzman RC. The hemochromatosis founder mutation in HLA-H disrupts β_2 -microglobulin interaction and cell surface expression. *J Biol Chem*. 1997;272:14025–14028.
19. Zhou XY, Tomatsu S, Fleming R, Parkkila S, Waheed A, Jiang J, Fei Y, Brunt EM, Ruddy DA, Prass CE, Schatzman RC, O'Neill R, Britton RS, Bacon BR, Sly WS. *HFE* gene knockout produces mouse model of hereditary hemochromatosis. *Proc Natl Acad Sci U S A*. 1998;95:2492–2497.
20. Feder JN, Penny DM, Irrinki A, Lee VK, Lebron JA, Watson N, Tsuchihashi Z, Sigal E, Björkman PJ, Schatzman RC. The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. *Proc Natl Acad Sci U S A*. 1998;95:1472–1477.
21. Waheed A, Parkkila S, Zhou XY, Tomatsu S, Tsuchihashi Z, Feder JN, Schatzman RC, Britton RS, Bacon BR, Sly WS. Hereditary hemochromatosis: effects of C282Y and H63D mutations on association with β_2 -microglobulin, intracellular processing, and cell surface expression of

- the HFE protein in COS-7 cells. *Proc Natl Acad Sci U S A*. 1997;94:12384–12389.
22. Tuomainen T-P, Salonen R, Nyssönen K, Salonen JT. Cohort study of relation between donating blood and risk of myocardial infarction in 2682 men in eastern Finland. *BMJ*. 1997;314:793–794.
 23. Salonen JT, Tuomainen T-P, Salonen R, Lakka TA, Nyssönen K. Donating blood is associated with a reduced risk of myocardial infarction: the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD). *Am J Epidemiol*. 1998;148:445–451.
 24. Salonen JT. The role of iron as a cardiovascular risk factor. *Curr Opin Lipidol*. 1993;4:277–282.
 25. Salonen JT. Body iron stores, lipid peroxidation and coronary heart disease. In: Hallberg L, Asp N-G, eds. *Iron Nutrition in Health and Disease*. London, UK: JL London Press; 1996:293–301.
 26. Smith C, Mitchinson MJ, Aruoma OI, Halliwell B. Stimulation of lipid peroxidation and hydroxyl-radical generation by the contents of human atherosclerotic lesions. *Biochem J*. 1992;286:901–905.
 27. Swain J, Gutteridge JM. Prooxidant iron and copper, with ferroxidase and xanthine oxidase activities in human atherosclerotic material. *FEBS Lett*. 1995;368:513–515.
 28. Lee FY, Lee TS, Pan CC, Huang AL, Chau LY. Colocalization of iron and ceroid in human atherosclerotic lesions. *Atherosclerosis*. 1998;138:281–288.
 29. Ambrosio G, Zweier JL, Jacobus WE, Weisfeldt ML, Flaherty JT. Improvement of postischemic myocardial function and metabolism induced by administration of deferoxamine at the time of reflow: the role of iron in the pathogenesis of reperfusion injury. *Circulation*. 1987;76:906–915.
 30. Farber NE, Vercellotti GM, Jacob HS, Pieper GM, Gross GJ. Evidence for a role of iron-catalyzed oxidants in functional and metabolic stunning in the canine heart. *Circ Res*. 1988;63:351–360.
 31. Nassar BA, Zayed EM, Title LM, O'Neill BJ, Bata IR, Kirkland SA, Dunn J, Dempsey GI, Tan MH, Johnstone DE. Relation of HFE gene mutations, high iron stores and early onset coronary artery disease. *Can J Cardiol*. 1998;14:215–220.
 32. Franco RF, Zago MA, Trip MD, ten Cate H, van den Ende A, Prins MH, Kastelein JJ, Reitsma PH. Prevalence of hereditary haemochromatosis in premature atherosclerotic vascular disease. *Br J Haematol*. 1998;102:1172–1175.
 33. Adams PC. Prevalence of abnormal iron studies in heterozygotes for hereditary hemochromatosis: an analysis of 255 heterozygotes. *Am J Hematol*. 1994;45:146–149.
 34. Bulaj ZJ, Griffen LM, Jorde LB, Edwards CQ, Kushner JP. Clinical and biochemical abnormalities in people heterozygous for hemochromatosis. *N Engl J Med*. 1996;335:1799–1805.
 35. Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, Strohmeyer G. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. *N Engl J Med*. 1985;313:1256–1262.
 36. Sullivan JL. Heterozygous hemochromatosis as a risk factor for premature myocardial infarction. *Med Hypotheses*. 1990;31:1–5.