# Clinical manifestations of hemochromatosis in *HFE* C282Y homozygotes identified by screening

Gordon D McLaren MD[1] Christine E McLaren PhD[2], Paul C Adams MD[3], James C Barton MD[4], David M Reboussin PhD[5], Victor R Gordeuk MD[6], Ronald T Acton PhD[7], Emily L Harris PhD MPH[8], Mark R Speechley PhD[9], Phyliss Sholinsky MSPH[10], Fitzroy W Dawkins MD[6], Beverly M Snively PhD[5], Thomas M Vogt MD[11], John H Eckfeldt MD PhD[12]; for the Hemochromatosis and Iron Overload Screening (HEIRS) Study Research Investigators

**GD McLaren, CE McLaren, PC Adams, et al; for the Hemochromatosis and Iron Overload Screening Study Investigators (HEIRS). Clinical manifestations of hemochromatosis in *HFE* C282Y homozygotes identified by screening. Can J Gastroenterol 2008;22(11):923-930.**

**BACKGROUND:** Patients with hemochromatosis may suffer organ damage from iron overload, often with serious clinical consequences.
**OBJECTIVE:** To assess prevalences of self-reported symptoms and clinical signs and conditions in persons homozygous for the hemochromatosis gene (*HFE*) mutation (C282Y) identified by screening.
**METHODS:** Participants were adults 25 years of age or older enrolled in the Hemochromatosis and Iron Overload Screening (HEIRS) Study. C282Y homozygotes (n=282) were compared with control participants without the *HFE* C282Y or H63D alleles (ie, wild type/wild type; n=364).
**RESULTS:** Previously diagnosed C282Y homozygotes and newly diagnosed homozygotes with elevated serum ferritin levels had higher prevalences of certain symptoms such as chronic fatigue (OR 2.8; 95% CI 1.34 to 5.95, and OR 2.0; 95% CI 1.07 to 3.75, respectively), and had more hyperpigmentation on physical examination (OR 4.7; 95% CI 1.50 to 15.06, and OR 3.7; 95% CI 1.10 to 12.16, respectively) and swelling or tenderness of the second and third metacarpophalangeal joints (OR 4.2; 95% CI 1.37 to 13.03, and OR 3.3; 95% CI 1.17 to 9.49, respectively) than control subjects. Joint stiffness was also more common among newly diagnosed C282Y homozygotes with elevated serum ferritin than among control subjects (OR 2.7; 95% CI 1.38 to 5.30). However, the sex- and age-adjusted prevalences of self-reported symptoms and signs of liver disease, heart disease, diabetes and most other major clinical manifestations of hemochromatosis were similar in C282Y homozygotes and control subjects.
**CONCLUSIONS:** Some symptoms and conditions associated with hemochromatosis were more prevalent among C282Y homozygotes identified by screening than among control subjects, but prevalences of most outcomes were similar in C282Y homozygotes and controls in this primary care-based study.

**Key Words:** *Complications; Cross-sectional study; Hemochromatosis;* HFE; *Iron overload; Prevalence*

## Les manifestations cliniques de l'hémochromatose chez des homozygotes C282Y *HFE* repérés par dépistage

**HISTORIQUE :** Les parents atteints d'hémochromatose peuvent souffrir d'une atteinte des organes causée par surcharge en fer, entraînant souvent de graves conséquences cliniques.
**OBJECTIF :** Évaluer la prévalence de symptômes déclarés par les patients ainsi que des signes cliniques et des pathologies des personnes homozygotes à la mutation (C282Y) du gène d'hémochromatose (*HFE*) repérées par dépistage.
**MÉTHODOLOGIE :** Les participants étaient des adultes de 25 ans et plus qui participaient à l'étude HEIRS sur le dépistage de l'hémochromatose et de la surcharge en fer. On a comparé les homozygotes C282Y (n=282) aux sujets témoins sans allèles C282Y ou H63D *HFE* (c'est-à-dire type sauvage/type sauvage; n=364).
**RÉSULTATS :** Les homozygotes C282Y déjà diagnostiqués et les homozygotes nouvellement diagnostiqués ayant des taux élevés de ferritine sérique présentaient une plus forte prévalence de certains symptômes, tels que la fatigue chronique (RRR 2,8; 95 % IC 1,34 à 5,95, et RRR 2,0; 95 % IC 1,07 à 3,75, respectivement), plus d'hyperpigmentation à l'examen physique (RRR 4,7; 95 % IC 1,50 à 15,06 et RRR 3,7; 95 % IC 1,10 à 12,16, respectivement) et un œdème ou une sensibilité des deuxième et troisième articulations métacarpophalangiennes (RRR 4,2; 95 % IC 1,37 à 13,03 et RRR 3,3; 95 % IC 1,17 à 9,49, respectivement) que les sujets témoins. La raideur articulaire était également plus courante chez les homozygotes C282Y nouvellement diagnostiqués ayant une ferritine sérique élevée que chez les sujets témoins (RRR 2,7; 95 % IC 1,38 à 5,30). Cependant, la prévalence de symptômes déclarés par le patient et de signes de maladie hépatique, de maladie cardiaque, de diabète et de la plupart des autres principales manifestations cliniques d'hémochromatose, rajustés selon le sexe et l'âge, était similaire chez les homozygotes C282Y et les sujets témoins.
**CONCLUSIONS :** Certains symptômes et certaines pathologies associés à l'hémochromatose étaient plus prévalents chez les homozygotes C282Y repérés par dépistage que chez les sujets témoins, mais la prévalence de la plupart des issues était similaire chez les homozygotes C282Y et les sujets témoins dans le cadre de la présente étude menée en soins primaires.

[1]*Department of Veterans Affairs Long Beach Healthcare System, Long Beach, and Division of Hematology/Oncology, Department of Medicine, University of California, Irvine;* [2]*Epidemiology Division, Department of Medicine, University of California, Irvine, California, USA;* [3]*Department of Medicine, London Health Sciences Centre, London, Ontario;* [4]*Southern Iron Disorders Center, Birmingham, Alabama;* [5]*Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, North Carolina;* [6]*Department of Medicine, Howard University, Washington DC;* [7]*Departments of Microbiology, Medicine, Genetics and Epidemiology and International Health, University of Alabama at Birmingham, Alabama;* [8]*Kaiser Permanente Center for Health Research, Portland, Oregon; current affiliation: Population Genomics, National Human Genome Research Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA;* [9]*Department of Epidemiology and Biostatistics, University of Western Ontario, London, Ontario;* [10]*Epidemiology Branch, National Heart Lung and Blood Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland;* [11]*Kaiser Permanente Center for Health Research, Honolulu, Hawaii;* [12]*Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota, USA*
*Correspondence and reprints: Dr Gordon D McLaren, VA Long Beach Healthcare System, Hematology/Oncology Section (11/111-H), 5901 East 7th Street, Long Beach, California, 90822 USA Telephone 562-826-8000 ext 4145, fax 562-826-5515, e-mail gordon.mclaren@va.gov*
*Received for publication April 27, 2008. Accepted July 31, 2008*

  

Hemochromatosis is an autosomal recessive disorder characterized by an increased risk of iron overload attributable to excessive dietary iron absorption (1-3). Left untreated, progressive iron overload can damage the liver, heart, pancreas, anterior pituitary gland and joints (1-5). Hemochromatosis is common among Caucasian populations of northern European origin. In the United States, 80% to 90% of Caucasian patients diagnosed with hemochromatosis are homozygous for a mutation in the hemochromatosis (*HFE*) gene (exon 4, nt845G→A; cys282tyr, C282Y) (6,7). Homozygosity for the C282Y mutation is found in approximately five of every 1000 persons of northern European descent (8-10). A second common mutation of *HFE* (exon 2, nt187C→G; his63asp, H63D) is more widely distributed in other populations; C282Y/H63D compound heterozygotes comprise a proportion of hemochromatosis patients (3) but exhibit milder phenotypic manifestations (2,9,11).

The major complications of iron overload in hemochromatosis patients can be prevented by phlebotomy therapy to remove excess iron, and patients treated before the onset of organ damage have a normal life expectancy (12). This has stimulated interest in early detection (3,13,14) and recent studies (15-19) have suggested that manifestations of severe iron overload are relatively uncommon in C282Y homozygotes identified by screening.

In the current study, we compared the prevalence of symptoms and clinical conditions among non-Hispanic Caucasian C282Y homozygotes identified in the multicentre Hemochromatosis and Iron Overload Screening (HEIRS) Study with the prevalence among control subjects from the same population. We hypothesized that symptoms and clinical conditions typically associated with iron overload are more prevalent in previously diagnosed C282Y homozygotes and in newly diagnosed C282Y homozygotes with elevated serum ferritin (SF) levels than in control participants. The investigators also postulated that the prevalence of symptoms and clinical conditions in newly diagnosed C282Y homozygotes with normal SF levels is not greater than in control participants.

## METHODS

### Subjects

The HEIRS Study is evaluating the prevalence, genetic and environmental determinants, and potential clinical, personal and societal impact of hemochromatosis and iron overload in a multiethnic, primary care-based sample of 101,168 adults enrolled over a two-year period at four field centres in the United States and one in Canada. Participants, who were at least 25 years of age and able to give informed consent, were recruited from a health maintenance organization, diagnostic blood collection centres, and public and private primary care offices and ambulatory clinics associated with the field centres (20). Initial screening of participants included genotyping for the *HFE* C282Y and H63D alleles, measurement of serum iron and unsaturated iron binding capacity, calculated transferrin saturation (TfS) and SF levels (20).

### Clinical examination

The study protocol was approved by the institutional review board of each field centre and informed written consent was obtained for screening and for a clinical examination (CE). The CE was offered to all *HFE* C282Y homozygotes and to all participants whose TfS and SF values exceeded study thresholds (TfS of more than 50% and SF more than 300 µg/L for men; TfS more than 45% and SF more than 200 µg/L for women) (20). Participants were also informed of their genotype, TfS and SF results, and at that time were invited to return for the CE. The median length of time between initial screening and the CE was approximately eight months; participants generally received their results several months before the CE. Of 2265 participants invited for CEs, 1687 (75%) participated. Among C282Y homozygotes (n=333), the participation rate was 91% (n=302), of whom 94% (n=285) were non-Hispanic Caucasians. A control group of 642 participants who carried neither the C282Y nor H63D *HFE* alleles (ie, wild type), and had SF and TfS levels between the 25th and 75th percentiles of sex-specific distributions, also had CEs. Of these, 366 were non-Hispanic Caucasians. Control subjects were frequency-matched for age and sex to cases studied at each field centre. Three C282Y homozygotes lacked SF measurements and two controls reported a previous diagnosis of iron overload or hemochromatosis and were excluded from the analyses. Among the non-Hispanic Caucasian C282Y homozygotes, three study groups were designated: previously diagnosed participants, newly diagnosed participants with elevated SF (SF level of more than 300 µg/L for men or more than 200 µg/L for women) and newly diagnosed participants without elevated SF (Table 1). The group of previously diagnosed C282Y homozygotes included those who indicated that they had been diagnosed with hemochromatosis or iron overload before enrolling in the HEIRS study or had undergone therapeutic phlebotomy either before enrolling in the study or before returning for the CE.

The CE has been described in detail previously (20) and this included a participant-completed questionnaire addressing medical history and a focused physical examination of the heart, liver, spleen, skin and metacarpophalangeal (MP) joints. The medical history questionnaire and physical examination were designed to document the prevalence of the kinds of symptoms and clinical conditions that have been reported in previous studies to be associated with hemochromatosis and iron overload (1-5,13). Examiners were aware of each participant's genotype, TfS and SF results at the time of the examination. Participants also completed the Short Form 36 (SF-36) Health Survey (21). In total, 36 outcome variables (symptoms, clinical conditions and physical signs) were included in this evaluation. A morning fasting blood sample was obtained for confirmation of genotyping results for the C282Y and H63D alleles (6,22), repeat TfS and SF determinations (20,23) and measurements of serum glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), C-reactive protein (CRP) (Hitachi 911 analyzer, Roche Applied Science, USA), and serum insulin (DPC Immulite Analyzer, Diagnostic Products, USA). If the ALT concentrations were elevated, testing for hepatitis B surface antigen and hepatitis C antibody was performed (Vitros ECi, Ortho-Clinical Diagnostics Inc, USA).

### Statistical methods

Binary outcomes were analyzed using logistic regression adjusted for sex, age and field centre, as appropriate. Some binary outcomes were positive too rarely to support reliable estimation and these were analyzed using Fisher's exact test for group effects without covariate adjustment. The SF-36 Health Survey was analyzed using analysis of covariance with covariate adjustment. The natural log transform was applied to fasting serum glucose, insulin, ALT, AST and GGT levels.

Transformed variables were analyzed using multiple linear regression. Elevated CRP (more than 5.0 mg/L) was analyzed as a binary outcome. Primary analyses focused on the F test for effects among all study groups. A Bonferroni correction for multiple comparisons was applied to control the type 1 error rate at 0.05 for 36 signs and symptoms and, separately, for six laboratory tests. Pairwise comparisons among the study groups were made using estimated ORs or least-squares means. For signs and symptoms, a subset of outcomes for which results may be biased as a result of exclusion of controls with SF levels greater than the 75th percentile (246 µg/L for men and 121 µg/L for women) were specified; the outcomes that were designated as possibly subject to bias in comparisons between C282Y homozygotes and controls are indicated in Table 2.

## RESULTS

### Study population

The initial screening of participants included 63,550 women (62.8%) and 37,618 men (37.2%). The median age was 50 years (range 25 to 100 years). By self-identified race/ethnicity, 44% of the participants were non-Hispanic Caucasians. As previously reported (24), the frequency of homozygosity for the C282Y mutation in this group of non-Hispanic Caucasians was 4.4 per 1000 subjects; consistent with the findings of other screening studies (9,18,25-29). Of 285 non-Hispanic Caucasians homozygous for C282Y who attended the CE, 282 had complete data for analysis. There were 195 newly diagnosed homozygotes (122 [63%] women and 73 [37%] men) of median age 51 years (range 25 to 86 years). Among them, 131 (67%; 67 [51%] women and 64 [49%] men) had elevated SF (more than 300 µg/L in men, more than 200 µg/L in women) at the time of CE, and 64 (33%; 55 [86%] women and 9 [14%] men) had SF below these levels. Eighty-seven other participants with C282Y homozygosity reported they previously were told by a physician that they had hemochromatosis or iron overload, or had been treated by therapeutic phlebotomy, including 39 (45%) women and 48 (55%) men, of median age 56 years (range 25 to 83 years). The characteristics of the groups of C282Y homozygotes and control participants are shown in Table 1. Because the previously diagnosed group included participants who had undergone phlebotomy therapy before initial screening or had begun phlebotomy treatments before returning for CE, clinical manifestations were evaluated in this group without regard to SF levels. Control subjects (n=364) were comprised of 222 (61%) women and 142 (39%) men, of median age 56 years (range 25 to 92 years).

**TABLE 1**
**Characteristics of non-Hispanic Caucasian C282Y homozygotes and control participants**

| | C282Y homozygotes | | | |
|---|---|---|---|---|
| | | Newly diagnosed | | |
| Characteristics | Previously-diagnosed (n=87) | Ferritin elevated* (n=131) | Ferritin not elevated† (n=64) | Control participants (n=364) |
| Age, years | 57.4±13.5 | 51.7±13.6 | 45.7±10.3 | 56.3±14.1 |
| Age range, years, n (%) | | | | |
| 24 to 44 | 14 (16) | 40 (30) | 27 (42) | 75 (21) |
| 45 to 64 | 47 (54) | 65 (50) | 36 (56) | 180 (49) |
| 65 and older | 26 (30) | 26 (20) | 1 (2) | 109 (30) |
| Women, n (%) | 39 (45) | 67 (51) | 55 (86) | 222 (61) |
| Transferrin saturation, % | 63.4±25.9 | 75.6±20.4 | 51.0±27.1 | 28.5±9.1 |
| Serum ferritin, µg/L‡ | 186.0 (58.0, 470.0) | 616.0 (428.0, 911.0) | 90.0 (32.0, 132.0) | 84.0 (50.5, 138.0) |

*Data presented as mean ± SD, unless otherwise indicated. *Serum ferritin >200 µg/L for women, >300 µg/L for men; †Serum ferritin ≤200 µg/L for women, ≤300 µg/L for men; ‡Serum ferritin results presented as median (25th percentile, 75th percentile)*

### Symptoms, clinical conditions, physical signs and biochemical test results

Table 2 shows the proportions of participants who reported clinical symptoms or had abnormal findings on physical examination. After adjustment for imbalances in age and sex, statistically significant differences between C282Y homozygotes and controls were observed for the following five (of 36) outcomes: symptoms of chronic fatigue/weakness, unexplained weight loss, joint stiffness or pain, and findings on physical examination of swollen or tender MP joints and increased pigmentation. Although the observed prevalences of most other outcomes were higher in previously diagnosed C282Y homozygotes, and to a lesser extent, in newly diagnosed homozygotes with elevated SF than in controls, there were no statistically significant differences for these outcomes. There also were no significant differences among the three groups of C282Y homozygotes for any symptom, sign or clinical condition. The only significant differences were between groups of C282Y homozygotes and controls (Table 3). In contrast, there were significant differences in serum ALT between groups of C282Y homozygotes (Table 4), with higher levels in previously diagnosed homozygotes and in newly diagnosed homozygotes with elevated SF than in newly diagnosed homozygotes with normal SF (P<0.001). However, the prevalences of self-reported liver disease, porphyria cutanea tarda and hepatomegaly on physical examination were not significantly different among groups of C282Y homozygotes and control subjects (Table 2). Of 76 participants with elevated ALT or AST, none tested positive for hepatitis B surface antigen and four tested positive for hepatitis C antibody, including one of 14 previously diagnosed C282Y homozygotes and three of 32 controls (data not shown). There was no significant difference among any of the groups in levels of fasting serum glucose, insulin, AST, GGT or prevalence of elevated CRP (Table 4).

Among symptoms with significantly different prevalences between C282Y homozygotes and controls, chronic fatigue/weakness was more prevalent both in previously diagnosed C282Y homozygotes and in newly diagnosed homozygotes with elevated SF. Self-reported unexplained weight loss was significantly more common among all groups of C282Y homozygotes than among controls, none of whom reported any unexplained weight loss. The SF-36 Health Survey score revealed no statistically significant differences among any of the groups (Table 2).

The prevalences of symptoms possibly related to heart disease, such as self-reported shortness of breath or ankle swelling, were not significantly different among any of the subgroups of C282Y homozygotes and control subjects (Table 2). There also was no significant difference in the prevalence of possible signs of heart disease such as frequent premature contractions, other rhythm disturbance or ankle edema determined by physical examination.

As shown in Table 2, self-reported joint stiffness was more common among C282Y homozygotes than in controls, and the prevalence was highest for newly diagnosed C282Y homozygotes with elevated SF, although a history of arthritis

**TABLE 2**
**Proportions of C282Y homozygotes and control participants with symptoms and signs**

| Symptoms and clinical conditions | C282Y homozygotes | | | Control participants (n=364) | Nominal P ‡ |
|---|---|---|---|---|---|
| | | Newly diagnosed | | | |
| | Previously diagnosed (n=87) | Ferritin elevated*(n=131) | Ferritin not elevated† (n=64) | | |
| General manifestations | | | | | |
| Chronic fatigue/weakness in the last 12 months§ | 51.7 | 39.7 | 34.4 | 23.6 | <0.001 |
| Unexplained weight loss in the last 12 months § | 5.8 | 6.9 | 3.1 | 0.0 | <0.001 |
| Health Survey score (mean)§ | 60.3 | 70.1 | 66.8 | 69.5 | 0.066 |
| Cardiac symptoms | | | | | |
| Swelling of feet or ankles in the last 12 months§ | 41.4 | 37.4 | 32.8 | 34.1 | 0.70 |
| Shortness of breath when walking quickly or uphill | 69.0 | 62.6 | 65.6 | 61.3 | 0.62 |
| Shortness of breath when walking on level ground | 27.6 | 12.2 | 20.3 | 12.1 | 0.033 |
| Shortness of breath while resting in a chair | 6.9 | 3.8 | 6.3 | 2.5 | 0.61 |
| Shortness of breath in the last 12 months | 41.4 | 29.8 | 31.3 | 23.6 | 0.10 |
| Heart failure or weak heart, ever | 9.2 | 1.5 | 3.1 | 5.2 | 0.33 |
| Abnormal heart rhythm, heart beat or action arrhythmia, ever | 23.0 | 15.3 | 18.8 | 21.2 | 0.74 |
| Other heart disease or heart attack, ever | 10.3 | 8.4 | 7.8 | 11.0 | 0.41 |
| Diabetes | | | | | |
| Diabetes, ever | 16.1 | 9.9 | 10.9 | 8.5 | 0.57 |
| Liver disease | | | | | |
| Abdominal swelling or fluid in last 12 months§ | 16.1 | 11.5 | 20.3 | 8.8 | 0.42 |
| Unexplained abdominal pain or discomfort in the last 12 months§ | 23.0 | 16.8 | 28.1 | 11.3 | 0.037 |
| Liver disease, ever§ | 16.1 | 9.2 | 7.8 | 6.0 | 0.094 |
| Porphyria cutanea tarda, ever§ | 4.6 | 0.8 | 1.6 | 0.3 | 0.038 |
| Arthritis | | | | | |
| Joint stiffness/pain/ache in last 12 months§ | 73.6 | 74.1 | 70.3 | 55.5 | <0.001 |
| Arthritis, ever§ | 57.5 | 35.1 | 29.7 | 38.7 | 0.084 |
| Neurological symptoms | | | | | |
| Unexplained confusion or memory loss in last 12 months | 27.6 | 16.8 | 18.8 | 9.1 | 0.078 |
| Hypogonadism | | | | | |
| Trouble having an erection or loss of sexual drive in last 12 months (men only) | 56.3¶ | 44.4** | 44.4†† | 36.6‡‡ | 0.023 |
| Other endocrine | | | | | |
| Thyroid disease, ever | 16.1 | 15.3 | 21.9 | 19.8 | 0.93 |
| Skin changes | | | | | |
| Change in skin colour in last 12 months§ | 12.6 | 10.7 | 12.5 | 3.0 | 0.0015 |
| Bone disease | | | | | |
| Osteoporosis, ever | 8.1 | 3.8 | 7.8 | 7.1 | 0.51 |
| Other | | | | | |
| Cancer§ | 10.3 | 16.8 | 14.1 | 14.3 | 0.048 |
| Signs on physical examination | | | | | |
| Heart arrhythmia: bradycardia | 0.0 | 0.0 | 0.0 | 0.0§§ | 1.00 |
| Heart arrhythmia: tachycardia | 1.2 | 0.0 | 0.0 | 0.0§§ | 0.23 |
| Frequent premature contractions (>1 ectopic beat/min) | 6.9 | 3.1 | 3.1 | 1.7§§ | 0.049 |
| Other abnormal rhythm | 3.5 | 0.0 | 0.0 | 3.3§§ | 0.058 |
| Heart murmur | 8.1 | 6.1 | 7.8 | 10.2§§ | 0.51 |
| Edema§ | 11.5 | 7.6 | 6.3 | 15.5§§ | 0.73 |
| Hepatomegaly§ | 10.3 | 9.2 | 4.7 | 3.3§§ | 0.068 |
| Splenomegaly§ | 2.3 | 0.0 | 1.6 | 0.6§§ | 0.14 |
| Metacarpophalangeal joints swollen or tender§ | 21.8 | 13.7 | 3.1 | 5.0§§ | <0.001 |
| Increased pigmentation on sun-exposed or unexposed areas§ | 21.8 | 9.9 | 7.8 | 4.4§§ | <0.001 |
| Blistering, ulcers, scarring of sun-exposed skin§ | 3.5 | 2.3 | 0.0 | 2.5§§ | 0.64 |
| Hypertrichosis (excess hair growth in sun-exposed areas)§ | 0.0 | 1.5 | 1.6 | 0.3§§ | 0.17 |

**Serum ferritin > 200 µg/L for women, >300 µg/L for men. †Serum ferritin ≤200 µg/L for women, ≤300 µg/L for men. ‡P value for the group effect from logistic regression or Fisher's exact test. Nominal P values are compared with 0.00139 (0.05/36) to assess significance with a 5% type-1 error rate; §Outcomes possibly subject to bias in comparisons between C282Y homozygotes and controls; ¶Denominator = 48; **Denominator = 63; ††Denominator = 9; ‡‡Denominator = 142; §§Denominator = 362*

was not reported more frequently by C282Y homozygotes than control subjects. On physical examination, previously diagnosed and newly diagnosed C282Y homozygotes with elevated SF, but not newly diagnosed C282Y homozygotes with normal SF, had swollen MP joints more often than in controls (Table 3).

The prevalences of self-reported impotence in men, unexplained confusion or memory loss, and a change in skin colour

were not significantly different across subgroups of C282Y homozygotes. In contrast, there was a significantly higher prevalence of increased pigmentation detected on physical examination in previously diagnosed and newly diagnosed C282Y homozygotes with increased SF than in controls (Table 3). There was no significant difference between C282Y homozygotes and controls in the prevalence on physical examination of other skin changes such as blistering or hypertrichosis (Table 2).

The prevalences of self-reported diabetes mellitus, thyroid disease, osteoporosis and cancer were similar in C282Y homozygotes and controls (Table 2).

## DISCUSSION

Our results indicate that most symptoms and clinical manifestations associated with hemochromatosis were not statistically significantly more common among C282Y homozygotes identified by screening than among controls in this primary care population. In particular, the prevalences of certain serious clinical manifestations of hemochromatosis, such as symptoms or signs related to liver disease or heart disease, were not significantly higher in C282Y homozygotes than in controls after correction for multiple comparisons. The clinical consequences of advanced iron overload have been documented in previous studies (30), particularly in patients identified on the basis of symptoms or signs of organ damage. In contrast, participants in the large multicentre HEIRS Study were enrolled without regard to any clinical conditions. Our findings are consistent with the results of several single-centre and regional population- and primary care-based studies (15-18). A recent study of first-degree relatives of patients with known C282Y hemochromatosis also found a low prevalence of clinical manifestations in family members who were C282Y homozygotes or C282Y/H63D compound heterozygotes, despite evidence of iron overload in most cases (31). However, a recent meta-analysis (32), based mainly on case-control studies, found an increased risk of liver disease among C282Y homozygotes compared to wild type/wild type control subjects. In that study, hemochromatosis genotypes were not associated with an increased risk of diabetes mellitus, heart disease, arthritis, stroke or cancer in the overall analysis, although there was an increased risk of diabetes mellitus in C282Y homozygotes compared with controls among subjects of northern European descent.

We observed significant differences between groups of C282Y homozygotes and controls for five of 36 outcomes: symptoms of chronic fatigue/weakness, weight loss, joint stiffness or pain, and findings on physical examination of swollen or tender MP joints, and increased pigmentation. However, only C282Y homozygotes with elevated SF levels showed significant differences from controls for all five outcomes. Newly diagnosed homozygotes with normal SF (86% females) did not show significant differences from controls for most of these symptoms and conditions (Table 3). The prevalences of these symptoms and signs did not differ statistically among previously diagnosed C282Y homozygotes and newly diagnosed homozygotes, nor between newly diagnosed C282Y homozygotes with elevated SF and newly diagnosed homozygotes without SF elevation.

Several factors may have favoured increased or decreased prevalences of outcomes. It is possible that C282Y homozygotes identified by screening in a primary care population represent a relatively healthy subgroup of all C282Y homozygotes. A

**TABLE 3**
**Associations between study groups and medical conditions related to hemochromatosis**

| Symptoms and signs | OR (95% CI) |
|---|---|
| **General** | |
| Chronic fatigue/weakness last 12 months. | |
| Previously diagnosed | 2.8 (1.34 to 5.95) |
| Newly diagnosed with elevated serum ferritin | 2.0 (1.07 to 3.75) |
| Newly diagnosed with normal serum ferritin | 1.6 (0.66 to 3.68) |
| Controls | 1.0 |
| Arthritis | |
| Joint stiffness/pain/ache in last 12 months. | |
| Previously diagnosed | 1.9 (0.85 to 4.29) |
| Newly diagnosed with elevated serum ferritin | 2.7 (1.38 to 5.30) |
| Newly diagnosed with normal serum ferritin | 2.3 (0.98 to 5.63) |
| Controls | 1.0 |
| **Signs on physical examination** | |
| Metacarpophalangeal joints swollen or tender | |
| Previously diagnosed | 4.2 (1.37 to 13.03) |
| Newly diagnosed with elevated serum ferritin | 3.3 (1.17 to 9.49) |
| Newly diagnosed with normal serum ferritin | 1.0 (0.11 to 8.75) |
| Controls | 1.0 |
| Increased pigmentation on sun-exposed or unexposed areas | |
| Previously diagnosed versus controls | 4.7 (1.50 to 15.06) |
| Newly diagnosed with elevated serum ferritin | 3.7 (1.10 to 12.16) |
| Newly diagnosed with normal serum ferritin | 4.6 (0.87 to 24.25) |
| Controls | 1.0 |

*For signs and symptoms with a significant overall group effect by the Bonferroni multiple comparisons procedure, pairwise comparisons significant at the nominal level (α=0.05) are shown*

**TABLE 4**
**Results of serum biochemical tests in C282Y homozygotes and control participants**

| | C282Y homozygotes | | | | |
|---|---|---|---|---|---|
| | | Newly diagnosed | | | |
| Test* | Previously diagnosed (n=87) | Serum ferritin elevated† (n=131) | Serum ferritin not elevated‡ (n=64) | Control participants (n=364) | Nominal P§ |
| Fasting glucose, (mmol/L) | 5.61 (4.94, 6.22) | 5.22 (4.83, 5.61) | 5.05 (4.80, 5.52) | 5.16 (4.88, 5.66) | 0.062 |
| Fasting insulin, (pmol/L) | 76 (49, 97) | 56 (35, 83) | 56 (42, 76) | 56 (42, 80) | 0.20 |
| Alanine aminotransferase (U/L) | 20.0 (15.0, 30.0) | 22.0 (16.0, 33.0) | 16.0 (11.0, 20.0) | 19.0 (14.5, 25.0) | 0.0047 |
| Aspartate aminotransferase (U/L)) | 22.0 (18.0, 29.0) | 22.0 (19.0, 30.0) | 19.0 (16.0, 21.5) | 21.0 (17.0, 25.0) | 0.040 |
| Gamma-glutamyltransferase (U/L) | 25.0 (17.0, 37.0) | 23.0(15.0, 34.0) | 16.5 (12.0, 29.5) | 19.0 (13.0, 31.0) | 0.070 |
| C-reactive protein >5.0 mg/L, n (%) | 27 (31) | 41 (31) | 25 (39) | 106 (29) | 0.62 |

*Data are presented as median (25th percentile, 75th percentile) unless otherwise indicated. *Laboratory reference ranges: fasting glucose:* 3.3–6.38 mmol/L*; fasting insulin: 0–139 pmol/L; alanine aminotransferase: 0–31 U/L (women), 0–40 U/L (men); aspartate aminotransferase: 0–31 U/L (women), 0–37 U/L (men); gamma-glutamyltransferase: 7–33 U/L (women), 11–51 U/L (men); C-reactive protein: 0–5.0 mg/L. †Serum ferritin >200 µg/L for women, >300 µg/L for men; ‡Serum ferritin ≤200 µg/L for women, ≤300 µg/L for men; §P-value for the study group effect. Nominal P-values are compared with 0.0083 (0.05/6) to assess significance with a 5% type 1 error rate*

potential bias possibly favouring increased prevalence of morbidity among C282Y homozygotes is that, by design, the selection criteria for control participants required SF levels between the 25th and 75th percentiles to exclude participants with increased or decreased iron stores. Thus, some control subjects having liver disease or inflammatory disorders and an associated increase in SF levels (33) were excluded by this criterion. In addition, capture of the medical history and physical examination data was not masked to genotype information, and this may have led to some overprobing of anticipated findings in C282Y homozygotes or underprobing in controls. However, a lack of strong bias is suggested by the fact that only a few outcomes were significantly more common in homozygotes despite previous knowledge of genotype by participants and examiners.

The higher prevalence of self-reported chronic fatigue and unexplained weight loss in C282Y homozygotes than in controls in the current study is consistent with previous reports of symptoms in patients with hemochromatosis (1-4,13,28,34). Fatigue is commonly reported by patients diagnosed with hemochromatosis, especially in those having serum ferritin levels greater than 1000 µg/L (35). Weight loss in hemochromatosis patients has been attributed to diabetes mellitus, other endocrinopathy, cirrhosis of the liver or other complications of advanced iron overload typical of such patients. However, weight loss is infrequently reported by persons diagnosed to have hemochromatosis in screening programs, presumably because most of them do not have severe iron overload (13). The relatively low prevalence of weight loss reports among C282Y homozygotes in the current study is consistent with the low prevalence of severe iron overload observed in homozygotes in the HEIRS Study (24). Similarly, there was no significant difference between C282Y homozygotes and controls in the prevalence of most other self-reported symptoms commonly associated with hemochromatosis, including diabetes mellitus. The lack of association between *HFE* mutations and diabetes is consistent with the results of some (16,24,36,37), but not all (32,38) previous studies.

The greater prevalence of self-reported joint stiffness/pain and swollen or tender MP joints on physical examination in C282Y homozygotes than in controls in the current study may represent clinical expression of the arthropathy of hemochromatosis (1-4). Although we did not find a significantly higher prevalence of self-reported arthritis in C282Y homozygotes than in controls, arthritis is one of the most common clinical conditions in hemochromatosis patients and often is the first to develop (39-42). In the current study, the prevalence of joint stiffness or pain in newly diagnosed C282Y homozygotes with normal SF was similar to that in previously diagnosed C282Y homozygotes and newly diagnosed homozygotes with elevated SF (Table 2). This raises the possibility that arthralgia may be an early manifestation of hemochromatosis that precedes the development of iron overload (42).

Increased pigmentation in patients with hemochromatosis can be manifested as either a bronze or slate grey appearance (1,2,4,13), although its presence may be subject to interobserver variability. In the current study, increased pigmentation on physical examination was observed more often in previously diagnosed C282Y homozygotes and newly diagnosed homozygotes with elevated SF (but not in newly diagnosed homozygotes with normal SF) than in control participants, consistent with a relationship between this finding and the severity of iron overload.

The cardiac manifestations of hemochromatosis often resolve after treatment to remove excess iron by therapeutic phlebotomy, and this may account for our observation that the prevalence of cardiac symptoms among previously diagnosed C282Y homozygotes was not significantly increased in comparison with controls. Hepatic fibrosis, and occasionally cirrhosis, can also be reversed by iron removal (43), but cirrhosis in most cases is irreversible and is associated with an increased risk of primary hepatocellular carcinoma. We previously reported that male C282Y homozygotes in the HEIRS Study were more likely to report a history of liver disease at the time of the initial screening examination than were participants without *HFE* mutations (24). A recent longitudinal study from Australia also found that male C282Y homozygotes with a serum ferritin level higher than 1000 µg/L were more likely to report a history of liver disease than men without *HFE* mutations (35). Although we did not find a significantly increased prevalence of self-reported liver disease in C282Y homozygotes relative to controls in the current study, this could have been an effect of the selection of controls for the clinical examination or differential recall bias between the time of initial screening and the subsequent clinical examination. Hepatic fibrosis and cirrhosis in hemochromatosis patients can also be silent, and a recent report documents comparable prevalences of fibrosis and cirrhosis on liver biopsy in apparently healthy Australian C282Y homozygotes identified either by screening during health checks (19% fibrosis and 12% cirrhosis in males) or because there was a family history of hemochromatosis (23% and 6%, respectively) (40). In a Norwegian population-screening study, similar but slightly lower prevalences of fibrosis (11%) and cirrhosis (4%) were found in male C282Y homozygotes undergoing biopsy (44). In the current study, ALT levels were higher in previously diagnosed C282Y homozygotes and newly diagnosed homozygotes with elevated SF than in newly diagnosed homozygotes with normal SF. We previously reported that, of 11 C282Y homozygotes with elevated SF (497 µg/L to 5200 µg/L) in the HEIRS Study who underwent clinical liver biopsy, fibrosis was present in eight cases, including one with cirrhosis (45). Thus, some C282Y homozygotes had previously undiagnosed liver damage that was revealed by biopsy.

A possible interpretation of the relative lack of an increased prevalence of symptoms and signs among C282Y homozygotes in the current study may be that most homozygotes do not develop sufficient iron overload to cause symptoms and clinical manifestations (15-18), even though the majority have evidence of excess iron accumulation (46). Previous studies of hemochromatosis patients (47) in referral practice settings have shown that the risk of hepatic cirrhosis in C282Y homozygotes begins to increase when SF levels rise above 1000 µg/L, but this SF level was exceeded in only 29 of 131 newly diagnosed C282Y homozygotes with elevated SF in the current study (24). Thus, the prevalence of disease manifestations in C282Y homozygotes identified by screening in primary care is likely to be much less than in C282Y homozygotes receiving care in referral settings, such as practices specializing in the care of patients with liver disease (48). This was pointed out previously by Edwards et al (49), who found that patients with hemochromatosis diagnosed as a result of clinical symptoms tended to have more advanced iron overload and more severe disease manifestations than asymptomatic patients diagnosed by screening.

It has been estimated that 10% to 33% of C282Y homozygotes may eventually develop hemochromatosis-associated

morbidity (29). The results of the current study indicate that C282Y homozygotes identified by screening for hemochromatosis or iron overload in primary care do not have a significantly increased prevalence of most symptoms and clinical conditions typically associated with hemochromatosis at the time of screening. The traditional approach is to screen for hemochromatosis in patients with classic manifestations such as hepatic and cardiac complications, diabetes, gonadal dysfunction and arthritis. Our results suggest that using this traditional approach, a majority of C282Y homozygotes may remain undetected. Whether, or the rate at which, such individuals will develop clinical symptoms related to iron overload in the future is unknown (50). However, a longitudinal study (40) has shown that in the absence of therapeutic phlebotomy, a progressive increase in SF occurs in a majority of C282Y homozygotes. Three other longitudinal studies have assessed morbidity. One population-based study (51) of 23 C282Y homozygotes found no evidence of hemochromatosis-associated liver disease after 25 years, although three of these subjects died before they could be examined. In contrast, a similar study (52) of 10 C282Y homozygotes over a 17-year study period documented cirrhosis or fibrosis in three of six subjects who had liver biopsies. In the recent Australian study (35), iron overload-related disease was documented in 28.4% of 203 male C282Y homozygotes followed for an average of 12 years. Prospective longitudinal studies are needed to assess iron accumulation in C282Y homozygotes with initially normal SF levels and to evaluate treatment outcomes in patients undergoing therapeutic phlebotomy (29).

**ACKNOWLEDGEMENTS**: Participating HEIRS Study Investigators and Institutions: Field centres: Birmingham, AL – University of Alabama at Birmingham: Dr Ronald T Acton (Principal Investigator), Dr James C Barton (Co-Principal Investigator), Ms Deborah Dixon, Dr Susan Ferguson, Dr Richard Jones, Dr Jerry McKnight, Dr Charles A Rivers, Dr Diane Tucker and Ms Janice C Ware. Irvine, CA – University of California, Irvine: Dr Christine E McLaren (Principal Investigator), Dr Gordon D McLaren (Co-Principal Investigator), Dr Hoda Anton-Culver, Ms Jo Ann A Baca, Dr Thomas C Bent, Dr Lance C Brunner, Dr Michael M Dao, Dr Korey S Jorgensen, Dr Julie Kuniyoshi, Dr Huan D Le, Dr Miles K Masatsugu, Dr Frank L Meyskens, Dr David Morohashi, Dr Huan P Nguyen, Dr Sophocles N Panagon, Dr Chi Phung, Dr Virgil Raymundo, Dr Thomas Ton, Professor Ann P Walker, Dr Lari B Wenzel and Dr Argyrios Ziogas. London, Ontario, Canada – London Health Sciences Centre: Dr Paul C Adams (Principal Investigator), Ms Erin Bloch, Dr Subrata Chakrabarti, Ms Arlene Fleischhauer, Ms Helen Harrison, Ms Kelly Jia, Ms Sheila Larson, Dr Edward Lin, Ms Melissa Lopez, Ms Lien Nguyen, Ms Corry Pepper, Dr Tara Power, Dr Mark Speechley, Dr Donald Sun and Ms Diane Woelfle. Portland, OR and Honolulu, HI – Kaiser Permanente Center for Health Research, Northwest and Hawaii, and Oregon Health and Science University: Dr Emily L Harris (Principal Investigator), Dr Mikel Aickin, Dr Elaine Baker, Ms Marjorie Erwin, Ms Joan Holup, Ms Carol Lloyd, Dr Nancy Press, Dr Richard D Press, Dr Jacob Reiss, Dr Cheryl Ritenbaugh, Ms Aileen Uchida, Dr Thomas Vogt and Dr Dwight Yim. Washington, DC – Howard University: Dr Victor R Gordeuk (Principal Investigator), Dr Fitzroy W Dawkins (Co-Principal Investigator), Ms Margaret Fadojutimi-Akinsiku, Dr Oswaldo Castro, Dr Debra White-Coleman, Dr Melvin Gerald, Ms. Barbara W. Harrison, Dr Ometha Lewis-Jack, Dr Robert F. Murray, Dr Shelley McDonald-Pinkett, Ms Angela Rock, Dr Juan Romagoza and Dr Robert Williams. Central Laboratory: Minneapolis, MN – University of Minnesota and University of Minnesota Medical Center, Fairview: Dr John H Eckfeldt (Principal Investigator and Steering Committee Chair), Ms Susie DelRio-LaFreniere, Ms Catherine Leiendecker-Foster, Dr Ronald C McGlennen, Mr Greg Rynders, Dr Michael Y Tsai and Dr Xinjing Wang. Coordinating Centre Winston-Salem, NC Wake Forest University: Dr David M Reboussin (Principal Investigator), Dr Beverly M Snively (Co-Principal Investigator), Dr Roger Anderson, Ms Aarthi Balasubramanyam, Ms Elease Bostic, Ms. Brenda L. Craven, Ms Shellie Ellis, Dr Curt Furberg, Mr Jason Griffin, Dr Mark Hall, Mr Darrin Harris, Ms. Leora Henkin, Dr Sharon Jackson, Dr Tamison Jewett, Mr Mark D King, Mr. Kurt Lohman, Ms Laura Lovato, Dr Joe Michaleckyj, Ms Shana Palla, Ms Tina Parks, Ms. Leah Passmore, Dr Pradyumna D Phatak, Dr Stephen Rich, Ms. Andrea Ruggiero, Dr Mara Vitolins, Mr Gary Wolgast and Mr Daniel Zaccaro. NHLBI Project Office: Bethesda, MD – Ms Phyliss Sholinsky (Project Officer), Dr Ebony Bookman, Dr Henry Chang, Ms Kristianne Cooper, Dr Richard Fabsitz, Dr Cashell Jaquish, Dr Teri Manolio and Ms Lisa O'Neill. NHGRI Project Office Bethesda, MD — Dr Elizabeth Thomson. Dr Jean MacCluer, Southwest Foundation for Biomedical Research, also contributed to the design of the present study. We thank Wen-Pin Chen, MS and Lu Wu, MS for assistance with statistical analyses.

**FINANCIAL SUPPORT:** The HEIRS study was initiated and funded by NHLBI, in conjunction with NHGRI. The study was supported by contracts N01-HC-05185 (University of Minnesota), N01-HC-05186 (Howard University), N01-HC-05188 (University of Alabama at Birmingham), N01-HC-05189 (Kaiser Permanente Centre for Health Research), N01-HC-05190 (University of California, Irvine), N01-HC-05191 (London Health Sciences Centre), and N01-HC-05192 (Wake Forest University). Additional support was provided by the General Clinical Research Center, School of Medicine, University of California, Irvine, with funds provided by the National Center for Research Resources, 5M01RR 00827-29, U.S. Public Health Service; GCRC grant #M01-RR00032 (University of Alabama at Birmingham); and the Howard University General Clinical Research Center (GCRC) grant, M01-RR10284 and from the NHLBI and the Office of Minority Health UH1-HL03679-05. The work was independent of the funding.

## REFERENCES

1. Powell LW, Jazwinska E, Halliday JW. Primary iron overload. In: Brock JH, Halliday JW, Pippard MJ, Powell LW, eds. Iron Metabolism in Health and Disease. Philadelphia: WB Saunders, 1994:227-270.
2. Pietrangelo A. Hereditary hemochromatosis – a new look at an old disease. N Engl J Med 2004;350:2383-97.
3. Adams P, Brissot P, Powell LW. EASL International Consensus Conference on Haemochromatosis. J Hepatol 2000;33:485-504.
4. Gordeuk VR, McLaren GD, Samowitz W. Etiologies, consequences and treatment of iron overload. Crit Rev Clin Lab Sci 1994;31:89-133.
5. Tavill AS. Diagnosis and management of hemochromatosis. Hepatology 2001;33:1321-8.
6. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996;13:399-408.
7. Edwards CQ, Ajioka RS, Kushner JP. Hemochromatosis: A genetic definition. In: Barton JC, Edwards CQ, eds. Hemochromatosis: Genetics, Pathophysiology, Diagnosis and Treatment. Cambridge, United Kingdom: Cambridge University Press;2000:8-11.
8. Rochette J, Pointon JJ, Fisher CA, et al. Multicentric origin of hemochromatosis gene (*HFE*) mutations. Am J Hum Genet 1999;64:1056-62. [Erratum in Am J Hum Genet 1999;64:1491.].
9. Merryweather-Clarke AT, Pointon JJ, Jouanolle AM, Rochette J, Robson KJ. Geography of *HFE* C282Y and H63D mutations. Genet Test 2000;4:183-98.
10. Acton RT, Barton JC, Snively BM, et al. Geographic and racial/ethnic differences in *HFE* mutation frequencies in the Hemochromatosis and Iron Overload Screening (HEIRS) Study. Ethn Dis 2006;16:815-21.

11. Hanson EH, Imperatore G, Burke W. *HFE* gene and hereditary hemochromatosis: A HuGE review. Human Genome Epidemiology. Am J Epidemiol 2001;154:193-206.
12. Niederau C, Fischer R, Purschel A, Stremmel W, Haussinger D, Strohmeyer G. Long-term survival in patients with hereditary hemochromatosis. Gastroenterology 1996;110:1107-19.
13. 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.
14. Schmitt B, Golub RM, Green R. Screening primary care patients for hereditary hemochromatosis with transferrin saturation and serum ferritin level: systematic review for the American College of Physicians. Ann Intern Med 2005;143:522-36.
15. Asberg A, Hveem K, Thorstensen K, et al. Screening for hemochromatosis: High prevalence and low morbidity in an unselected population of 65,238 persons. Scand J Gastroenterol 2001;36:1108-15.
16. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of 845G → A (C282Y) *HFE* hereditary haemochromatosis mutation in the USA. Lancet 2002;359:211-8.
17. Asberg A, Hveem K, Kruger O, Bjerve KS. Persons with screening-detected haemochromatosis: As healthy as the general population? Scand J Gastroenterol 2002;37:719-24.
18. McCune CA, Ravine D, Carter K, et al. Iron loading and morbidity among relatives of *HFE* C282Y homozygotes identified either by population genetic testing or presenting as patients. Gut 2006;55:554-62.
19. US Preventive Services Task Force. Screening for hemochromatosis: Recommendation statement. Ann Intern Med 2006;145:204-8.
20. McLaren CE, Barton JC, Adams PC, et al. Hemochromatosis and Iron Overload Screening (HEIRS) study design for an evaluation of 100,000 primary care-based adults. Am J Med Sci 2003;325:53-62.
21. Ware JE, Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473-83.
22. Jeffrey GP, Chakrabarti S, Hegele RA, Adams PC. Polymorphism in intron 4 of *HFE* may cause overestimation of C282Y homozygote prevalence in haemochromatosis. Nat Genet 1999;22:325-6.
23. Adams PC, Reboussin DM, Leiendecker-Foster C, et al. Comparison of the unsaturated iron-binding capacity with transferrin saturation as a screening test to detect C282Y homozygotes for hemochromatosis in 101,168 participants in the hemochromatosis and iron overload screening (HEIRS) Study. Clin Chem 2005;51:1048-52.
24. Adams PC, Reboussin DM, Barton JC, et al. Hemochromatosis and iron-overload screening in a racially diverse population. N Engl J Med 2005;352:1769-78.
25. 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;318:1355-62.
26. Phatak PD, Sham RL, Raubertas RF, et al. Prevalence of hereditary hemochromatosis in 16031 primary care patients. Ann Intern Med 1998;129:954-61.
27. Olynyk JK, Cullen DJ, Aquilia S, Rossi E, Summerville L, Powell LW. A population-based study of the clinical expression of the hemochromatosis gene. N Engl J Med 1999;341:718-24.
28. Delatycki MB, Allen KJ, Nisselle AE, et al. Use of community genetic screening to prevent *HFE*-associated hereditary haemochromatosis. Lancet 2005;366:314-6.
29. Whitlock EP, Garlitz BA, Harris EL, Beil TL, Smith PR. Screening for hereditary hemochromatosis: A systematic review for the U.S. Preventive Services Task Force. Ann Intern Med 2006;145:209-23.
30. Ajioka RS, Kushner JP. Clinical consequences of iron overload in hemochromatosis homozygotes. Blood 2003;101:3351-3.
31. Watkins S, Thorburn D, Joshi N, et al. The biochemical and clinical penetrance of individuals diagnosed with genetic haemochromatosis by predictive genetic testing. Eur J Gastroenterol Hepatol 2008;20:379-83.
32. Ellervik C, Birgens H, Tybjaerg-Hansen A, Nordestgaard BG. Hemochromatosis genotypes and risk of 31 disease endpoints: Meta-analyses including 66,000 cases and 226,000 controls. Hepatology 2007;46:1071-80.
33. Worwood M. Determination of iron status In: Brock JH, Halliday JW, Pippard MJ, Powell LW, eds. Iron Metabolism in Health and Disease. Philadelphia: WB Saunders, 1994:449-76.
34. Swinkels DW, Aalbers N, Elving LD, Bleijenberg G, Swanink CM, van der Meer JW. Primary haemochromatosis: A missed cause of chronic fatigue syndrome? Neth J Med 2002;60:429-33.
35. Allen KJ, Gurrin LC, Constantine CC, et al. Iron-overload-related disease in *HFE* hereditary hemochromatosis. N Engl J Med 2008;358:221-30.
36. Acton RT, Barton JC, Bell DS, Go RC, Roseman JM. *HFE* mutations in African-American women with non-insulin-dependent diabetes mellitus. Ethn Dis 2001;11:578-84.
37. Acton RT, Barton JC, Passmore LV, et al. Relationships of serum ferritin, transferrin saturation, and *HFE* mutations and self-reported diabetes in the Hemochromatosis and Iron Overload Screening (HEIRS) study. Diabetes Care 2006;29:2084-9.
38. Salonen JT, Tuomainen TP, Kontula K. Role of C282Y mutation in haemochromatosis gene in development of type 2 diabetes in healthy men: Prospective cohort study. BMJ 2000;320:1706-7.
39. Adams PC, Chakrabarti S. Genotypic/phenotypic correlations in genetic hemochromatosis: evolution of diagnostic criteria. Gastroenterology 1998;114:319-23.
40. Powell LW, Dixon JL, Ramm GA, et al. Screening for hemochromatosis in asymptomatic subjects with or without a family history. Arch Intern Med 2006;166:294-301.
41. Adams PC, Kertesz AE, Valberg LS. Clinical presentation of hemochromatosis: A changing scene. Am J Med 1991;90:445-9.
42. Adams PC, Deugnier Y, Moirand R, Brissot P. The relationship between iron overload, clinical symptoms, and age in 410 patients with genetic hemochromatosis. Hepatology 1997;25:162-6.
43. Falize L, Guillygomarc'h A, Perrin M, et al. Reversibility of hepatic fibrosis in treated genetic hemochromatosis: a study of 36 cases. Hepatology 2006;44:472-7.
44. Asberg A, Hveem K, Halvorsen TB, Smethurst HB. Prevalence of liver fibrosis and cirrhosis in screening-detected C282Y homozygous subjects. Scand J Gastroenterol 2007;42:782-3.
45. Adams PC, Passmore L, Chakrabarti S, et al. Liver diseases in the hemochromatosis and iron overload screening study. Clin Gastroenterol Hepatol 2006;4:918-23.
46. Adams PC. Screening for haemochromatosis – producing or preventing illness? Lancet 2005;366:269-71.
47. Morrison ED, Brandhagen DJ, Phatak PD, et al. Serum ferritin level predicts advanced hepatic fibrosis among US patients with phenotypic hemochromatosis. Ann Intern Med 2003;138:627-33. [Erratum in Ann Intern Med 2003;139:235].
48. Bacon BR, Olynyk JK, Brunt EM, Britton RS, Wolff RK. *HFE* genotype in patients with hemochromatosis and other liver diseases. Ann Intern Med 1999;130:953-62.
49. Edwards CQ, Griffen LM, Kushner JP. The morbidity of hemochromatosis among clinically unselected homozygotes: Preliminary report. Adv Exp Med Biol 1994;356:303-8.
50. Qaseem A, Aronson M, Fitterman N, Snow V, Weiss KB, Owens DK. Screening for hereditary hemochromatosis: A clinical practice guideline from the American College of Physicians. Ann Intern Med 2005;143:517-21.
51. Andersen RV, Tybjaerg-Hansen A, Appleyard M, Birgens H, Nordestgaard BG. Hemochromatosis mutations in the general population: Iron overload progression rate. Blood 2004;103:2914-9.
52. 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;79:309-13.