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Screening for Hereditary Hemochromatosis:
A Focused Evidence Review

Investigators:
Evelyn P. Whitlock, MD, MPH
Betsy A. Garlitz, MD
Emily L. Harris, PhD, MPH
Tracy L. Beil, MS
Paula R. Smith, RN, BSN

Addendum to this Evidence Synthesis

An additional relevant study (Powell LW, Dixon JL, Famm GA, Purdie DM, Lincoln DJ, Anderson GJ, et.al. Screening for hemochromatosis in asymptomatic subjects with and without a family history. *Arch Intern Med* 2006;177:294-301) was published between the finalization of this evidence synthesis and the publication of the recommendation statement and peer-reviewed manuscript derived from this evidence synthesis in the *Annals of Internal Medicine.*

The findings from the Powell study are incorporated into key questions 1 and 2 in the published article, but not in this evidence synthesis. (*Annals* citation: Whitlock EP, Garlitz BA, Harris EL, Beil TL, Smith PR. *Screening for Hereditary Hemochromatosis: Systematic Review. Ann Intern Med* 2006;145:209-23. Also available at www.ahrq.gov/clinic/uspstf06/hemochromatosis/hemochrev.pdf.)

The USPSTF judged that the additional findings from this study confirm the earlier evidence and did not change its overall assessment of the evidence nor its recommendation statement.

Structured Abstract

Objectives: To assess evidence sufficiency or insufficiency for hereditary hemochromatosis screening relating to two main United States Preventive Services Task Force (USPSTF) criteria: the burden of suffering and the potential effectiveness of a preventive intervention.

Data Sources: MEDLINE®, CINAHL, and Cochrane Library databases from 1966 through February 2005. We supplemented literature searches with source materials from experts in the field and from examining the bibliographies of key reviews and included studies.

Review Methods: In conjunction with USPSTF leads and AHRQ staff, we developed three key questions with supporting definitions to capture the sufficiency of critical evidence necessary to make a recommendation for hereditary hemochromatosis as a new USPSTF screening topic. The critical evidence we reviewed to answer these questions focused on the development of disease in screen-identified C282Y homozygotes (penetrance), the incremental benefit of earlier therapeutic phlebotomy treatment, and whether there are high-risk groups for possible targeted genetic screening.

Using inclusion/exclusion criteria specific to each key question (KQ), we reviewed 1886 abstracts for inclusion in all key questions and 133 full-text articles for inclusion in KQ1, 67 articles for KQ2, and 55 articles for KQ3. Using USPSTF methods, we critically appraised studies using quality criteria specific to their design. To augment criteria provided for non-randomized treatment effectiveness studies, we added methods from the Cochrane Collaboration. We listed studies excluded from analysis and rationales for their exclusion. Our review abstracted, critically appraised, and synthesized 18 articles meeting our criteria for KQ1, six studies for KQ2, and 10 studies for KQ3.

Using pre-established condition definitions and screening and diagnostic criteria, we abstracted all studies into evidence tables. We summarized study results for disease development in those identified through two strategies, initial genotypic and initial phenotypic (biochemical) screening followed by genotypic screening.

Results: Disease expression or penetrance is less than 100% in C282Y homozygotes identified through some screening method, but data were insufficient to define a very precise estimate of penetrance. Although available data suggest that 38-50% of C282Y homozygotes develop iron overload and 10-25% develop some type of hemochromatosis-associated morbidity, current research represents very limited numbers of observations and research designs subject to bias. The incremental benefit of earlier therapeutic phlebotomy is logical but not well supported by the limited treatment evidence.

Conclusions: Research addressing genetic screening for hereditary hemochromatosis remains very limited. Not enough is yet known to confidently project the impact or benefit from widespread genetic screening for hereditary hemochromatosis.

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I. Introduction

Screening for Hereditary Hemochromatosis is a topic that has not been previously considered by the United States Preventive Services Task Force (USPSTF) for a possible recommendation as a clinical preventive service for primary care clinicians. This product is a pilot approach to systematically review the sufficiency of evidence for a focused (as opposed to comprehensive) set of key questions related to two main USPSTF screening criteria: the burden of suffering and the potential effectiveness of a preventive intervention, therapeutic phlebotomy.[1,2] The USPSTF considers conditions of major significance that are relatively common in the U.S. to be candidates for preventive interventions, such as screening.[2] Also, for a condition to be a candidate for a USPSTF screening recommendation, there must be evidence that persons with the condition detected early have a better clinical outcome that those detected without screening.[1] Key questions for this review were limited to what the USPSTF judged to be critical evidence gaps in establishing these requirements. Key questions were constructed and applied using strict and consistent definitions of disease, which are described in more detail below. The resulting report is intended to demonstrate evidence sufficiency or insufficiency as its primary aim, and thus does not have the usual breadth associated with a USPSTF systematic evidence review.

Condition Definition

Hemochromatosis (HC) was originally thought to be a rare idiopathic disorder characterized by end-stage disease (cirrhosis, diabetes, and bronzed skin), but is now recognized as often having a hereditary component due to an autosomal recessive inherited disorder of iron metabolism.[3] In HC, body iron accumulates and can lead to iron overload.[4] In iron overload, excess iron is deposited in the liver, pancreas, heart, joints, and endocrine glands, resulting in tissue damage that may lead to one or more disease conditions (e.g., cirrhosis, diabetes, increased skin pigmentation, heart failure, arthropathy, and impotence).[4-6] Iron overload can be primary (as in hereditary hemochromatosis) or secondary (e.g., due to anemias with inefficient erthropoeisis or repeated blood transfusions).[7]

Genetic understanding of hereditary hemochromatosis (HH) was advanced in 1996 when Feder identified two base pair alterations, termed C282Y and H63D, of the *HFE* gene on the region of *HLA-A* on chromosome 6 in hereditary hemochromatosis.[8] C282Y homozygosity is now recognized as the most common genotype in hereditary hemochromatosis.[9] Estimates are that 82 to 90% of HH among Caucasians occurs in C282Y/C282Y homozygotes.[10] The other 10 to 18% of cases appear to be due to environmental factors and/or other genotypes. There are other *HFE*- and non-*HFE*-related genetic mutations associated with HH in a small minority of cases,[4] but no other genotypes appear to be nearly as predisposed to developing HH as *HFE* C282Y/C282Y.[3,9]

HFE mutations are fairly common in the U.S. population. In Caucasians, 4.4 per 1000 are homozygous and 1 in 10 are heterozygous for (carriers of) the *HFE* C282Y mutation.[6,11] The frequency of C282Y homozygosity is much lower among Hispanics (0.27 in 1000), Asian Americans (<0.001 per 1000) and Pacific Islanders (0.12 per 1000), and Blacks (0.14 per 1000).[11] Individuals homozygous for the C282Y genotype can be characterized in one of four general stages: genetic predisposition without any other abnormality; iron overload without

symptoms; iron overload with early symptoms; iron overload with organ damage, especially cirrhosis.[4] The availability of genotyping has made it possible to identify those with the susceptible genotype with little or no evidence of disease.

Clinically recognized HH is twice as common in males and occurs predominantly in Caucasian populations.[12] While the natural history is not well understood, the condition appears to have a long latent period. Biochemical expression appears to vary widely among individuals,[13] and iron accumulation and disease expression are modified by environmental factors, such as blood loss from menstruation or donation, alcohol intake, diet, and comorbid disease (e.g. viral hepatitis).[14,15] If symptomatic organ involvement develops, it is generally in mid-life with non-specific signs and symptoms (e.g., unexplained fatigue, joint pain, abdominal pain).[3] Age of onset is delayed in females,[16] perhaps due to blood loss through menstruation.[3] The liver is the first target organ thought to be affected by iron accumulation,[17] which is central to both diagnosis and prognosis.[13] While a clinical diagnosis is sometimes based on serum iron studies and clinical evaluation, documentation of iron overload relies on one of two methods: quantitative phlebotomy (repeated phlebotomy to iron depletion) with calculation of the amount of iron removed; or liver biopsy with determination of quantitative hepatic iron.[18] Although liver biopsy was once essential to the diagnosis, it is currently used more as a prognostic tool.[19] A hepatic iron concentration above 283 micromoles per gram (reference range = 0 to 35 micromoles per gram dry weight) is associated with cirrhosis in C282Y homozygotes.[20] Many patients with much higher levels, however, do not have cirrhosis.[13] Even in the absence of systemic iron overload, iron accumulates when the liver is inflamed or cirrhosed due to other causes (e.g., alcoholic steatohepatitis, transfusion and chronic hemolytic disorders, or chronic viral hepatitis).[21]

Cirrhosis is a late-stage disease development and has been reported to shorten life expectancy.[22-25] Cirrhosis is also a risk factor for hepatocellular carcinoma[13] and typically occurs between the ages of 40 to 60 years.[6] Cirrhosis is less frequently a finding at diagnosis of clinical hemochromatosis than in the past,[26] and cirrhosis prevention would be a major goal of screening and treatment.[27]

Prevalence and Burden of Disease

The general population prevalence of HC is difficult to establish due to its long, variable preclinical period and to lack of a consistent "case" definition for disease. The prevalence of "cases of HC" defined as biochemical (elevated serum iron indices) will be more prevalent than cases based on documented iron overload, with or without clinical signs and symptoms; the lowest prevalence will be for cases based on diagnosed disease (cirrhosis, diabetes).[28] Experts have recommended defining an iron overload state as distinct from HC,[4] and this provides an objective, although not universally accepted, standard for "early disease" based on documented increases in body iron stores.[28]

Available population-based studies have generally used case definitions based on clinical diagnoses of HC (or diagnoses compatible with HC) to estimate the population-level disease burden for this condition. A total of 79,850 HH-associated hospitalizations (2.3 per 100,000 residents) were projected in the U.S. population over 18 years (1979 to 1997); annual rates could not be reliably calculated.[29] Out of 29 million deaths from 1979 to 1992, a total of 4858

(0.017%) were consistent with HC as an underlying cause.[12] Age-adjusted mortality rates for HC-consistent deaths increased from 1.2 per million U.S. residents in 1979 to 1.8 per million U.S. residents in 1992. These rates were about twice as high in males than females and in whites than non-whites. These two estimates were based on similar ICD-9 codes, but suggest a disease prevalence that is much lower than the prevalence of associated genetic mutations, which has fueled debate about disease penetrance (the proportion of those with the genotype expressing the phenotype) in HH. These statistics are probably underestimates, primarily due to underdiagnosis,[30] but the extent of this underestimation is not clear.

The prevalence of HC-attributable morbidities (e.g., cirrhosis, diabetes, arthralgias, and fatigue or other symptoms) may help estimate the size of the burden due to undiagnosed disease, particularly since diagnosis may commonly be delayed due to the non-specific nature of HC-related signs and symptoms.[31] However, since these signs and symptoms are also quite prevalent and non-specific to HC, relevant evidence must establish their prevalence due to iron overload or their excess prevalence in association with iron overload compared with controls. Recent general population and clinical population studies suggest some morbidities possibly associated with unrecognized HC are more prevalent in newly detected cases than would be expected. Compared with controls, 297 previously undetected, middle-aged genotypic hemochromatosis cases (HH: homozygous for C282Y), and 269 previously undetected, middle-aged phenotypic hemochromatosis cases (unselected HC: repeated high serum transferrin saturation (TS) and high serum ferritin (SF) without other known causes), had a higher prevalence of diagnosed osteoarthritis, knee complaints, hypothyroidism, and use of antihypertensive or thyroid replacement medications than sex- and age-specific controls.[32] However, general health, mental health, and 52 other questionnaire- and clinical-exam-based measures of cardiovascular, respiratory, and liver diseases were not statistically different in cases than controls. This study did not statistically adjust for multiple comparisons. In another cross-sectional comparison of 124 C282Y screen-detected adult homozygotes with 22,394 wild type/wild type genotypic controls, common symptoms (chronic fatigue, joint symptoms, impotence, limited general health) and signs (diabetes) were no more frequent in C282Y homozygotes than controls.[33] While the relative risk of doctor-diagnosed liver problems or hepatitis was increased (RR 2.1, 95% CI 1.1, 4.0), the proportion of C282Y homozygotes with liver problems was modest (10%). Similarly, in the HEIRS study, C282Y homozygotes had an increased odds of self-reported liver disease (3.28, 95% CI, 1.49, 7.22) compared with wild-type controls however, almost one-fourth were not newly identified.[11]

The meaning of population-based studies, such as these, for establishing the prevalence and disease burden, is debated, and other types of evidence are cited in the debate. Experts also argue over biases from observational studies, emphasizing problems with under-diagnosis, study populations used in available studies, estimates from uncontrolled studies, and patients selected for controlled comparisons.[34-40]

Rationale for Population Screening

Screening for HC or iron overload is theoretically attractive and has been widely discussed in the last 10 to 15 years, with renewed interest and a focus on HH since the discovery of the *HFE*

mutations.[4,41-44] Indeed, many have claimed that HH meets all the criteria for screening set forth by the USPSTF and other prominent organizations:[7,16,45-47] the genetic condition predisposing to iron overload is prevalent; treatment with periodic phlebotomy appears to be a safe and effective way to reduce progression in those with early disease; commonly available laboratory tests appear to be sensitive in identifying iron overload in its early phases (enhanced by availability after the mid-1990s of genotyping to identify HH); and little harm should result from a minimally invasive screening usually based on blood or other readily available tissues. Based on these factors, population screening for HH using biochemical (phenotypic screening) and/or genetic tests (genotypic screening) would appear to have a high net benefit, where the benefits substantially outweigh harms.

Although HH appears to be ideal for population screening[48] and a "new paradigm for genetics and public health,"[42] inadequacies in the evidence supporting genetic screening for HH have precluded wide-spread calls for population-based screening.[4,9,42,48]

A major source of uncertainty lies in the natural history and burden of illness, as introduced above, which must be understood as causing significant preventable disease in the U.S.[2] *How much disease is actually caused by HFE mutations?* This has been repeatedly identified as the most critical evidence issue preventing population screening, relating to whether sufficient data exist to determine the proportion of individuals identified through genetic testing who will develop clinically significant disease.[3,4,48]

Screening programs must be based on the benefits of early detection and treatment beyond the health improvement that would be seen if diagnosis and treatment occurred during the usual process of care.[2] To support population screening, treatment must not only be accessible, safe, cheap, and effective, but treatment initiated earlier than currently occurs after disease identification must also show clear benefit. The second major uncertainty lies in the evidence to support a benefit of early treatment. *Does therapeutic phlebotomy treatment, initiated through earlier identification of those with HH, lead to better outcomes?*

Focused Systematic Review Aims

In this focused systematic review, we addressed the adequacy of current evidence to answer the two critical uncertainties central to making primary-care-based screening for HH eligible for recommendation in the general population. We also considered the evidence supporting the identification of high-risk groups for screening.

Although there are multiple etiologies for both iron iron overload and HC, this review focused on the evidence regarding penetrance in hereditary disease and the extent and progression of disease in persons identified as having a HH susceptibility genotype. We focused on hereditary *HFE*-associated hemochromatosis due to C282Y homozygosity in persons of Northern European descent, which is the most prevalent form of HH in the United States. Other *HFE* and non-*HFE* genetic mutations are much rarer causes of hemochromatosis[49] and data for their disease association are more sparse than for C282Y homozygosity.[9] Thus, we did not think that the sufficiency of evidence for this topic would be enhanced by also considering other causes of HH, and their inclusion would only further complicate the issue.

II. Methods

Key Questions

In conjunction with US Preventive Services Task Force leads and Agency for Healthcare Research and Quality (AHRQ) staff, we developed three explicit questions (see Table 1) with supporting definitions (Table 2) to capture the critical evidence in making a recommendation for HH as a new screening topic. Key Question 1 examines the development of clinical hemochromatosis among C282Y/C282Y homozygotes (penetrance). Key Question 2 addresses intermediate and health outcomes of therapeutic phlebotomy treatment for asymptomatic primary iron overload due to HH, emphasizing the incremental benefit of earlier treatment. Key Question 3 examines whether there are identifiable groups at high-risk for developing HH for possible targeted genetic screening.

Literature Retrieval

We developed literature search strategies and terms for each Key Question (KQ) (Appendix A) and conducted four separate literature searches (for KQs 1, 2, 3, and background) in Medline, CINAHL, and the Cochrane library. Literature searches were supplemented with source material from experts in the field and from examining the bibliographies of included studies. A single investigator reviewed abstracts, with all excluded abstracts reviewed by a second investigator for all KQs. Inter-reviewer discrepancies during the dual review process were resolved by consensus.

Abstract/Article Review and Data Abstraction

Using inclusion criteria developed for each key question (described in Appendix A), we reviewed 1886 abstracts for inclusion in all KQs. Literature searches were focused for each KQ, but were reviewed with all KQs in mind. We reviewed 133 full-text articles for KQ1, 67 articles for KQ2, and 55 articles for KQ3. Two investigators quality rated all included articles and those excluded for quality-reasons, using the USPSTF criteria (Appendix B). Listings of excluded articles, with reasons, for each by KQ are in Appendices C, D, and E.

Critical Appraisal of Included Studies

Using methods of the USPSTF,[50] we critically appraised studies using quality criteria specific to their design (Appendix B). To augment criteria provided for non-randomized treatment effectiveness studies, we added criteria from the Non-Randomized Studies Methods Group of the Cochrane Collaboration.[51] We eliminated any case series or non-randomized comparative treatment study that used a non-systematic method of case ascertainment. We critically considered the comparability of constructed comparison groups, particularly concerning confounding factors (age, sex, alcohol intake, population prevalence of C282Y homozygosity, and comorbid liver disease) and secular trends in disease diagnosis and medical care. We eliminated those with possible serious biases.

Terminology and Definitions

We found very inconsistent uses of terminology regarding hemochromatosis in the literature, complicated by evolution in the understanding of its pathogenesis, hereditary components, and secondary causes over the last 20 to 30 years, and by disagreements among experts. We adopted the set of terms in Table 2 for use in this review.

Similarly, diagnostic and screening criteria also varied markedly. After reviewing several textbooks, the Hemochromatosis and Iron Overload Screening (HEIRS) study protocol, and the screening and diagnostic approaches used in several recent, good-quality studies, we established *a priori* screening and diagnostic criteria for elevated iron parameters and iron overload due to HH to guide our review and to establish comparability between studies (see Table 3).

Data Synthesis

We evaluated whether our review identified adequate data to create one or more outcomes tables to illustrate the expected yield from screening using an approach adapted from a previous report.[43] For this approach, we considered data for two different screening populations (general population and family-based) for genotypic screening followed by phenotypic screening and phenotypic screening followed by genotypic screening. Phenotypic screening approaches included TS (single or repeated) and SF measured singly, in sequence, or in combination. Genotypic screening required *HFE* genotyping and determination of C282Y homozygosity. Insufficient data across these parameters were available to create a reliable outcomes table for either screening approach. Few studies reported results for genotype, iron parameters, and iron overload, generating very small numbers for within-study morbidity estimates. We therefore summarized screening data in tables as described below.

Summary Tables. Data for family-based and general populations were kept separate. Data for the general population were categorized into subgroups and combined, where they were sufficiently similar. To evaluate the extent and adequacy of data for each parameter, and to combine data across studies, we selected data from studies included in our review to meet minimum *a priori* criteria (developed from the literature) for three parameters (Tables 2 and 3): 1) screen positive for elevated iron parameters; 2) documented iron overload; and 3) morbidity due to clinical hemochromatosis. For iron overload and morbidity, we calculated and reported two proportions (**selected** and **all**). Among those selected, we reported the positives among those who were actually tested for iron overload or morbidity (maximum penetrance) and second for all who screened positive in the first screening step (minimum penetrance). Within each screening approach, we evaluated whether results were similar enough to combine across studies and, when they were, we quantitatively combined study results for each parameter to generate a single point estimate for that parameter. Some general population subgroups were kept separate (blood donors). Other general population groups were summarized within categories (health clinics, voter roles, and employee screening) and across categories (all combined as general population). We reported a range of results for any parameter for which individual study results were too different to be meaningfully combined. We did not include individual study results with 10 or fewer subjects in the denominator to define a range, but did include these results if

they could be combined with other results in a single parameter estimate. Study results were reported as raw numbers for denominators of 10 or less.

External Review Process

The USPSTF appointed four liaisons to advise the Oregon Evidence-based Practice Center in formulating and reporting this focused systematic review. An additional set of outside experts provided advice in the review formulation stage and gave us comments on a draft version of the evidence synthesis.

III. Results

Key Question 1. Among those with a homozygous C282Y genotype, what is the risk of developing clinical hemochromatosis?

Out of 133 full-text studies examined for both of these questions, we excluded 115 studies for reasons specified by our inclusion/exclusion criteria (Appendix C Table 2.). We eliminated all studies that reported on morbidity in a mixed source population (persons identified from more than one of family, clinical, and healthy populations) using combined measures only, due to potential differences in disease expression between these groups. We eliminated studies that did not report actual morbidities associated with clinical hemochromatosis (or at least iron overload) among participants. Other primary reasons for study exclusions included reporting on samples that did not derive from any population—particularly one that could be subject to screening— and for reporting study data that could not fit within our hemochromatosis-related definitions. Two studies were identified but not published at the time of this report (Appendix C Table 3).

Summary of findings. The best evidence available came from longitudinal studies reporting the risk of developing disease in initially undiseased persons. Cross-sectional studies were more plentiful, but provide an estimate of disease expression only at the time of examination.

Although there are no longitudinal studies in inception cohorts, two fair-to-good quality retrospective cohort studies from Australia[52] and Denmark[53] report on disease expression (penetrance) of 33 C282Y homozygotes (22 women and 11 men) over 17 to 25 years of follow-up. Participants' average age at the end of observation was 47 to 63 years, however, eight females were 50 years or younger at final follow-up. This represents 8 of 33 (24%) of those observed who may not yet have reached an age to manifest clinical expression. Most (61 to 75%), but not all, of participants had elevated serum iron parameters by the end of observation. In the Australian study only, iron overload was objectively evaluated by liver biopsy in six of 10 participants. Iron overload was detected in five of 10 participants in the study. Considering both studies together, two of 33 developed diabetes and six of 33 had arthralgias. By clinical examination, none of the 23 Danish patients had liver disease, cardiomyopathy, or hypogonadism. Considering the two studies together, liver disease is clearly present in 3/33. Progression of iron accumulation did not appear inevitable, particularly as measured by SF, since a number of individual patients showed decreases or no change in SF levels over time, despite a lack of treatment or plausible explanation such as blood donation or loss.

In 13 cross-sectional studies, C282Y homozygotes were identified through three health clinics,[33,54-58] in blood donor settings,[59-61] through mass screening,[62] through voter rolls or employment screening,[63-66] or through family screening.[67,68] Disease expression at the time of identification was available for C282Y homozygotes identified through non-family-based genetic screening of 67,771 patients or from genotyping over 200 family members of probands. Seven cross-sectional studies provide adequate data to estimate the prevalence of iron overload and disease at the time of screening in the general population.[33,56,62-66] Among a total of 228 not previously identified C282Y homozygotes, 38% of those further evaluated met criteria for iron overload, 25% had liver fibrosis, and 6% had cirrhosis. These estimates could be too high (if the untested C282Y homozygotes are not as likely to be penetrant) or too low (if the other untested C282Y homozygotes are more likely to be penetrant) and should be viewed with caution as they are based on very small numbers. Limited data from genotyping family members of probands were consistent with a higher proportion of iron overload (49 to 86%) or cirrhosis (8%) in C282Y homozygotes identified through family screening than from general population screening.

Individual longitudinal studies. (Table 4) A good-quality retrospective cohort study examined biochemical and clinical study data gathered over the prior 17 years in 10 of 12 newly identified C282Y homozygotes (six females; four males) from genotyping the surviving Busselton, Australia cohort in 1998.[52] By the median age of 43, nine of 10 had TS above 45%, and five of 10 had SF levels above 300 µg/L. All subjects showed increased TS over time, while SF increased in four patients, stayed the same in four patients, and decreased in two patients. Decreases could not be explained by blood loss or donation. By the median age of 47 years, six of 10 patients had SF levels above 500 µg/L and they underwent liver biopsy. Five of six patients biopsied had hepatic iron concentrations above 90 micromoles/gram (moderate iron overload according to HEIRS standards). One of six patients had cirrhosis, and that person reported alcohol intake above six drinks per day. Two other patients had fibrosis and one of 10 had diabetes unrelated to HH. Comparative data were not provided for other genotypes. Selective mortality bias could have influenced these findings to represent the healthier homozygotes, although the C282Y/C282Y prevalence was 5.3 per 1000, and data were completed on 83% of the sample. However, how well these data represent life-long disease penetrance is in question. Two reports of the same data suggest different ages for participants at final follow-up. Based on a previous report of the same data, five females were 50 years or under at follow-up, while the other five C282Y homozygotes were males over 40 years or females over 50 years.[62]

A fair-quality retrospective and prospective cohort study, originally selected to represent the general population of Denmark, genotyped 9174 individuals at their third examination in 1991 to 1994 (65% of the original sample).[53] Twenty-three individuals were C282Y homozygotes (0.25%) and were matched according to age, sex, and alcohol consumption to two individuals with two other genotypes: wild type/wild type; compound heterozygote (C282Y/H63D). At the 2001 study examination, which included examination by a hemochromatosis specialist, 20 of 23 were still alive. In 2001 (or the last study examination for patients who had died), 9 of 16 C282Y homozygote females and 5 of 7 C282Y homozygote males met biochemical screening criteria for iron overload (TS > 45% and SF > 200 µg/L in females; TS > 50% and SF > 300 µg/L in males). At their last contact, female patients' mean age was 63.5 years, and 3 of 16 were

50 years old or younger, 7 male patients had a mean age of 61.8 years, and none were 40 years old or younger. C282Y homozygotes tended to have higher average TS and SF than other genotypes, with the exception of SF in males. Not all homozygotes, however, showed an increase in iron parameters over time and iron parameter levels showed more substantial individual variation over time than other genotypes. One of 23 C282Y homozygotes (male, age 45 years) was diagnosed with sub-clinical hemochromatosis. In 2001, no C282Y homozygotes had evidence of liver disease, based on symptoms and biochemical tests (no biopsies), and 1 patient had insulin-treated diabetes. Other hemochromatosis signs or symptoms (hypogonadism, cardiomyopathy, arthritis) were rare. Selective mortality bias may be a concern, as 35% of the original sample was not genotyped and the prevalence of C282Y homozygosity in those remaining to be tested was relatively low (0.25%). The authors state "the C282Y/C282Y genotype frequencies found in the remaining cohort did not differ from that predicted by the Harvey-Weinberg equilibrium." Also, 3 of 23 patients died before genotyping (none from apparent hemochromatosis-related causes). However, the upper bound of morbidity can be calculated for disease penetrance. If all 3 patients among the 23 patients died from hemochromatosis, the proportion developing hemochromatosis-related disease would still be at about one-quarter (4/23). If the 35% of the cohort lost to follow-up had the usual population prevalence of C282Y homozygosity (5/1000), then about 25 C282Y homozygotes would have been lost to follow-up. If all of them fully developed clinical disease, then penetrance would be 60% (29/48) at the time of follow-up.

Individual cross-sectional studies. Morbidity findings in newly identified C282Y homozygotes from health clinics (n=50,434), the general population (3011), and voter roles and employee screening settings (14,326) are detailed in Appendix C Table 1. We kept three blood donor studies (n=16,842) separate and used them to estimate the lower bound of elevated iron parameters in C282Y homozygotes identified through general population genetic screening. Blood donors were not used to estimate iron overload or morbidities in C282Y homozygotes due to lack of data and because disease prevalence could be affected by donor policies (identifying and eliminating known C282Y homozygotes) and disease expression could be affected by the treatment effect of frequent blood donation. Two family screening studies reported iron parameters along with morbidity in 25 C282Y homozygotes identified from screening 150 family members[67] and of iron overload according to hepatic criteria in 51 C282Y homozygotes identified from screening an unspecified number of family members.[68]

The similarity of findings for C282Y prevalence and phenotypic screening in health clinics, general population, voter roles, and employee screening studies allowed them to be considered together as "general population" (Table 5). The prevalence of C282Y homozygosity was 4.2 per 1000 in the general population (Table 6), 2.5 per 1000 in blood donors, and 161 per 1000 in family screening. Estimates for the prevalence of positive results for elevated TS and SF were relatively consistent among combined general population and blood donor studies, except for the broad range of results for elevated TS in general population females (40 to 94%). As might be expected, elevated TS estimates in blood donors tended towards the lower end of the general population range (56 to 70%) and in family members towards the higher end of the range (87.5%). SF levels were elevated in 54 to 58% of females and 58 to 76% of general population males, with slightly lower proportions in blood donors and elevated proportions (96%) in family members, an overall pattern similar to TS.

Iron overload was documented in 38% (range 24 to 75%) of 69 general population C282Y homozygotes selected for further evaluation. This translates to 24% (range 13 to 46%) of all C282Y homozygotes, assuming the absence of iron overload in those not further evaluated. One of two studies in family members reported iron overload in 51 C282Y homozygote family members.[68] Among 51 family members of probands, 86% of those evaluated (49% of all C282Y homozygote family members) had iron overload by hepatic criteria. The proportion of family members genotyped that were positive for C282Y homozygosity was not reported and so this study did not qualify to be reported in our summary tables. The other family study that qualified for summary tables did not report on iron overload in family members.[67]

The prevalence of cirrhosis or fibrosis could be estimated for 72 C282Y homozygotes identified from the general population[62,65,66] and 25 C282Y homozygotes from family screening.[67] One of 16 patients biopsied had cirrhosis, while four had fibrosis. A minimum of 8% (2/25) of family-identified C282Y homozygotes had cirrhosis; fibrosis wasn't reported. In the general population, 0 to 5.6% of C282Y homozygotes had diabetes mellitus; one study reported a higher prevalence of diabetes mellitus (8.4%) in matched patients with non-homozygous genotypes.[33] Diabetes mellitus was present in 16% of C282Y homozygotes identified from families. In comparison to C282Y/C282Y family members, no compound heterozygote family members (C282Y/H63D, n=23) had hepatic cirrhosis of diabetes at the time of screening.[67]

Key Question 2. Does earlier therapeutic phlebotomy of individuals with primary iron overload due to HH reduce morbidity and mortality compared with treatment after diagnosis in routine clinical care?

We found no controlled studies of phlebotomy treatment in patients with hemochromatosis due to any cause, nor any studies that allowed comparison of early vs. delayed treatment. Three fair-quality case series of hemochromatosis patients reported objective measures before and after, or simply after, treatment[25,26,69] in six publications.[22,23,25,26,69,70] One retrospective observational survey [71] reported changes in symptoms after treatment among hemochromatosis patients identified through multiple outreach mechanisms (Appendix D Table 1). We excluded 61 other full-text articles, primarily due to study quality, size (fewer than 20 patients), or not containing primary data or relevant outcomes (Appendix D Table 2).

Summary of findings. Available treatment studies (three fair-quality case series of referral center patients) report on the survival experience of 447 total patients and the reduction in morbidity after treatment of 350 HC patients identified and treated over a 50-year period after 14.1 (±6.8) mean years of follow-up.[25,26,69] Only 85 of these patients had genotypically confirmed HH. Survival in diagnosed HC patients has improved over time and 10-year survival data in recently diagnosed patients does not differ from age- and sex- matched population controls. Similarly, survival in HH patients without cirrhosis at diagnosis does not differ from survival in population controls. Cirrhosis at diagnosis appears to confer a worse prognosis, although studies do not compare patients that are clearly similar. Comparisons of patients with less severe and more severe disease are biased by secular trends in disease severity at diagnosis, and perhaps secular differences in hemochromatosis treatment. Pre- and post-treatment liver

biopsies suggest less reversibility in cirrhosis once it develops, compared with less severe liver disease, although these data come from uncontrolled observational studies. Some, but not all, symptoms appear to respond to treatment. Secular trends raise questions about the study relevance of most existing treatment studies and complicate their interpretation.

Individual studies. 336 patients from the two largest case series did not clearly have HH (i.e., and were identified over very long time periods 1937 to 1975[69] and 1947 to 1991.[26] Another smaller study reported on 85 HH cases (56% probands and 44% family members), identified from 1958 to 1989.[25] Cumulative survival at five years was 87 to 93%, at 10 years was 77 to 81%, and at 20 years was 55 to 71%,[25,26] which was decreased compared with age-and-sex matched population controls. Fewer patients with HH had cirrhosis at diagnosis (32%), compared with unselected hemochromatosis patients (57 to 79%). Secular trends in disease severity and survival, however, were apparent over the time period these studies transpired (Figure 1). Those diagnosed in 1982 to 1991 showed better survival over 10 years of follow-up than the two groups diagnosed earlier (log-rank test, p≤0.05), and cumulative survival for HC patients diagnosed in 1982 to 1991 was not significantly reduced from rates expected for an age- and sex-matched population.[26] There was no significant difference, however, between survival in patients without cirrhosis at diagnosis and population controls.[25] Survival differences between patient subgroups (e.g., cirrhotic vs. non-cirrhotic, diabetics vs. non-diabetics),[25,26] or between all patients and historical controls,[69] were also reported, but are not reliable due to potential confounding by uncontrolled and unmeasured factors, such as time period of diagnosis, age at diagnosis, sex, excessive alcohol use, concomitant hepatitis, and dietary factors.

Response to treatment was gauged by comparing pre-post symptoms and clinical features, including level of fibrosis on biopsy, for 185 unselected HC patients without regard to time of diagnosis.[26] Given the secular trends in symptoms and disease severity, the most meaningful data are those based on objective changes in liver biopsies by degree of fibrosis at baseline. Degree of fibrosis was: Stage 0 (pre-fibrosis, non-fibrosis, only septal fibrosis); Stage 1 (nonextensive portal fibrosis without bridging septa); Stage 2 (portal fibrosis with briding septa); Stage 3 (annual fibrosis with vascular disruption and cirrhosis). About half of patients with post-treatment repeat liver biopsies (n=93) had Stage 3 fibrosis/cirrhosis at diagnosis; 12 (13%) of these improved to Stage 2 fibrosis after treatment, none worsened, and 81 (87%) were unchanged. Among patients with Stage 2 fibrosis (n=39), about half (n=20) improved to Stage 1, none worsened, and about half (n=19) were unchanged. Among those with Stage 1 fibrosis (n=32), one-third showed reversal to pre-fibrosis (Stage 0), one worsened to Stage 2, and 21 (66%) were unchanged. Among those with no fibrosis at baseline (Stage 0), most (20/21) were unchanged, while one patient worsened to Stage 1 fibrosis. Very few patients' liver biopsies showed more severe fibrosis and liver biopsies after treatment, and liver pathology was unchanged for most patients, suggesting that treatment arrested disease progression. However, there is no untreated control group with which to compare these findings. For those with some level of liver fibrosis before treatment, 13 to 50% showed some improvement, with the lowest proportional improvement seen in those with the most advanced liver disease (Stage 3), suggesting that treatment is more effective in earlier disease. These findings, however, are based on qualitative histological readings and masking in outcome assessment was not clearly employed.

Cirrhosis (as opposed to fibrosis or normal liver) was significantly related to the risk of death in treated HH patients (adjusted relative risk 5.54, 95% C.I., 1.76, 17.47 (calculated)), controlling for arthritis and age at diagnosis.[25] This analysis did not control for date of diagnosis (occurring over 31 years) and presented sparse information on other possible confounders, such as alcohol intake.

Post-treatment liver biopsies showed no histological changes in 68 of 75 patients (91%), of whom 56 had cirrhosis and 12 had portal fibrosis, improvement from cirrhosis to portal fibrosis in five patients (7%), and progression from portal fibrosis to cirrhosis in two patients (3%).[69] Twelve patients (16%) accumulated enough iron after treatment to require a repeat course of venesection. The study reported comparisons to untreated historical controls (n=26), but these were excluded due to non-comparability between the two groups and potential confounding by secular trends in treatment improvements for severe disease, such as diabetes mellitus or hepatic failure.

One fair-quality retrospective and prospective case series assessed changes in nine signs and symptoms associated with HH six months after completion of phlebotomy treatment for biopsy-proved iron depletion in 183 primary care patients in Germany, diagnosed between 1947 and 1991.[26] Most (89%) were male, had cirrhosis or diabetes (57% and 48%), and were symptomatic at diagnosis. Six reasonably clear criteria for change were reported. Insulin-dependent diabetes mellitus was present in 25% of patients (46/183) and the daily insulin dose could be reduced in 41% of patients (19/46). Of 148 patients with elevated liver enzymes (ALT or AST), 108 (73%) showed improvement. Symptoms such as weakness/lethargy or abdominal pain were reported as absent after treatment in more than half of patients, which was not observed for arthralgias or loss of potency (30% and 19% resolved, respectively). Response to treatment was gauged by comparing pre- and post-symptoms and clinical features for patients regardless of time of diagnosis. Sign and symptom changes reported here are from an extremely heterogeneous group due to the secular trends in symptoms and disease severity at this study's presentation.

Response to treatment through retrospective patient recall was assessed in a large-scale study that attempted to survey known hemochromatosis patients in the United States (and to a lesser extent, Australia, U.K., and Canada) through direct mailings and solicitations through hemochromatosis-related organizations.[71] Respondents (2851/3562 mailings, 80%) were overwhelming white (99%), mostly male (62%), and had primarily been diagnosed after 1990 (70%). Most patients (65%) were diagnosed through family screening or an abnormal lab test. The frequency of several hemochromatosis-associated conditions (arthritis, diabetes mellitus, liver or gallbladder disease extreme fatigue) in U.S. survey respondents was quite similar to the frequency in the U.S. general population using NHANES data, with some excess reported in younger HC patients. Patients were asked to recall symptoms associated with their disease and the proportion that improved with therapy. Eighty-six percent of patients reported some or all symptoms improved with therapy, although one-third (33%) reported developing new symptoms despite therapy. Extreme fatigue improved in more than half of patients with that symptom, while depression and abdominal pain improved in 41% and 22%, respectively. Few reported improvements in impotence (13%) or joint pain (9%). How well these survey respondents represent all patients is not clear, because all responses were based on recall, and primary and secondary hemochromatosis did not appear to be distinguished (although the method of

recruitment should have primary HC and HH). The absence of controls removes the possibility of assessing for a placebo effect or comparing non-specific symptom prevalence and changes over time.

Key Question 3. Are there group(s) at increased risk for developing HH that can be readily identified prior to genetic screening?

We examined 55 full-text articles and excluded 45 studies from this question for reasons (Appendix E Table 2), such as not reporting relevant measures or results, addressing the wrong population, not using C282Y genotype to define the family risk group, non-included study designs, and quality. Two fair-to-good quality cross-sectional studies of family members and seven fair-to-good quality cross-sectional studies (in eight publications) of patients with signs or symptoms met our inclusion criteria. For family history risk determination, we included studies with C282Y-genotyped probands.[67,72] Besides family members of known C282Y homozygotes, other at-risk groups included patients with increased liver enzymes, fatigue, cardiac pacemakers, and those from specialty liver, endocrinology, and rheumatology clinics.[56,73-79] (See Appendix E Table 1 for full evidence table).

Summary of findings. Groups evaluated for increased risk of clinical hemochromatosis due to a higher prevalence of C282Y homozygosity included 434 family members of probands and 42,868 patients with signs or symptoms consistent with, but not specific for, iron overload taken from primary care or specialty settings. Family screening strategies identify the highest prevalence of undetected C282Y homozygotes (13 to 49%), particularly among siblings of probands and, perhaps, family members of clinically detected (as opposed to screen-detected) probands. Some patients selected for a variety of hemochromatosis-related symptoms and diseases have a higher prevalence of C282Y homozygosity compared with controls. Very selected chronic fatigue and arthralgia patients and hospitalized diabetic patients have a higher prevalence of C282Y homozygosity (5.7 to 5.8%), which is further increased (6.6 to 18.6%) when only those with elevated iron parameters are genotyped. Liver patients with elevated iron parameters also have an increased C282Y homozygous prevalence (7.1%). On the other hand, primary care patients selected for symptoms or signs consistent with HC have the same prevalence of C282Y homozygosity as controls, as do other types of selected patients. All null findings should be viewed with particular caution since many comparisons involved fewer than 250 patients per group which may seriously limit power to demonstrate a difference in prevalence since C282Y homozygosity in the general population is three to five per 1000.

Individual family-based studies. The prevalence of C282Y homozygosity among family members of probands is considerably higher than in the general population, but varies by how the probands were identified and their relationship to the family member. Among parents and adult siblings of HH probands (all C282Y/C282Y), 49% (59/121) of the relatives of those identified clinically were also C282Y homozygotes, compared with 15% (25/163) of the relatives of C282Y/C282Y probands identified from screening blood donors.[72] Fifty-nine percent of the 34 previously untested C282Y homozygous relatives of clinically identified probands had elevated iron parameters, as did 40% of the 25 homozygous relatives of screen-detected probands. However, these percentages may not be comparable as it is not clear what proportion of these 25 relatives were newly diagnosed. A higher proportion (53%) of relatives of

clinically-identified probands had already been tested than of screen-detected probands (24%). Although 92% of eligible relatives of screen-detected probands and 85% of eligible relatives of clinically-identified probands participated, these data may not be entirely representative, as already-identified persons may have been more or less likely to participate. Among 112 first-, second-, and third-degree relatives of 61 unselected HC patients (73.8% of whom were C282Y homozygotes), 22.3% were C282Y homozygotes.[67] Siblings had the highest prevalence of C282Y homozygosity (33%), compared with parents, offspring, and other biological relatives (15.7%).

Individual studies in symptomatic patients. (Individual study details in Appendix E Table 1) A fair-quality, cross-sectional cohort study conducted after 1996 in France provided the most comprehensive data on the prevalence of the C282Y homozygous genotype among symptomatic patients.[75] Patients were selected from five different health care settings: 1) primary care (n=169 patients with an index sign or symptom for hemochromatosis); 2) rheumatology clinics (n=221 Rheumatoid factor negative patients with osteoporosis or arthropathy); 3) endocrinology clinics (n=121 diabetic patients hospitalized for diabetes-related complications); 4) referral medicine clinics (n=227 patients referred with chronic fatigue/arthralgia); and 5) 991 controls, a random sample of subjects attending a health appraisal clinic. The proportion of patients homozygous for C282Y ranged from 0 to 5.8% in various clinical populations, compared with 0.2% in the healthy volunteers. No difference in the prevalence of C282Y homozygosity was seen between controls or primary care patients with an index sign or symptom. C282Y homozygosity was significantly more prevalent in hospitalized diabetic patients from an endocrinology clinic (5.8%) and in referral medicine clinic patients with chronic fatigue and arthralgias (5.7%) (p<0.001) than in controls. In other studies, the prevalence of C282Y homozygosity appeared the same (women), or slightly higher (0.57% vs. 0.28% in men), in patients from a health appraisal clinic with elevated liver enzymes compared to those with normal enzymes.[56] Males, but not females, from the same clinic with chronic fatigue symptoms had a slightly higher (0.85%) prevalence of C282Y homozygosity, compared with those without symptoms (0.14%). Patients with a history of coronary heart disease appear to have the same or lower prevalence of C282Y homozygosity (0.17 to 0.28%) than those without symptoms.[79] Of 232 pacemaker patients, three had biopsy-proven iron overload, but *HFE* genotype was not available and HLA-typing showed only one-third to be consistent with genetic hemochromatosis.[76] Rheumatology patients appear to have a C282Y homozygosity prevalence similar to the general population.[78]

A higher prevalence of C282Y homozygosity may be found in some groups of symptomatic patients when restricted to those who also have elevated iron parameters (Table 7). In 667 patients referred for investigation of liver disease, the prevalence of new HH cases by phenotypic screening was 2.8%; among those liver patients with increased TS levels (above 45%), 7.1% were homozygous for C282Y.[73] For hospitalized patients with diabetes and patients with chronic fatigue or arthralgias that were referred to specialists, C282Y homozygosity was higher in patients with TS above 40% and/or SF > 300 μg/L than in diseased patients without elevated iron measures (6.6% to 18.6% compared with 5.7% to 5.8%). The sensitivity of TS > 40% for detecting C282Y homozygosity in diabetics hospitalized for disease-related complications was 100%, but the specificity was 13%. In diabetic patients, the sensitivity of SF > 300 μg/L was 86% and the specificity was 56%. For patients referred for arthralgias and unexplained fatigue, TS > 40% and SF > 300 μg/L were about equally sensitive and specific for C282Y homozygosity

(100% sensitive and 65 to 67% specific). Data are too sparse in rheumatology patients prescreened with elevated serum iron measures[75] and in a study of 88 patients with chronic fatigue syndrome.[77]

IV. Discussion

Natural History/Burden of Disease
(Key Question 1)

We considered C282Y homozygotes identified through genotyping general population groups separately from those identified through genotyping family members of a known C282Y homozygote, to allow for differences in other factors affecting disease development (penetrance).

In the general population, two fair-to-good quality population-based cohort studies reported the risk of developing signs or symptoms of iron overload and hemochromatosis in 33 C282Y homozygote adults monitored for 17 to 25 years. Another 13 fair-to-good quality cross-sectional studies reported on the burden of disease at the time of identification for an additional 228 newly identified C282Y homozygote adults identified from the general population. Taken together these data suggest that 38 to 50% of C282Y homozygotes develop iron overload meeting our criteria and 10 to 25% develop definite disease. These data do not establish estimates of disease penetrance as they represent very small numbers of people (10 total C282Y homozygotes from longitudinal studies), and disease development could still occur in persons with longer-follow-up. Indeed, eight of 33 of those followed longitudinally were females age 50 or under at last follow-up, in whom disease may not have yet developed. And, it should be kept in mind that the clinical significance of iron overload is less clear than that of clinical hemochromatosis.

In family members of probands, there are no longitudinal studies reported of C282Y homozygotes identified through screening family members, but two cross-sectional studies suggest that the prevalence of C282Y homozygosity in family members is higher, as is the disease burden, compared with C282Y homozygotes identified through general population screening means. These data may not be very precise, however, due to potential selection biases and uncontrolled observations. Equally limited data suggest 49 to 86% of C282Y homozygotes from family screening will meet iron overload criteria and 8% will have cirrhosis. These data support the current clinical practice of genotyping and phenotyping family members of probands to identify and treat affected members. There are other ethical, legal, social, and psychological issues associated with family screening, however.[80]

Benefits of Early Treatment
(Key Question 2)

Therapeutic phlebotomy studies examining survival and morbidity after treatment are limited to three case series reporting on a total of 447 patients diagnosed between 1937 and 1989. Two

studies provide additional data on response to treatment. Disease severity at diagnosis and survival showed pronounced secular trends over this time period. More recently diagnosed patients are less-severely affected and show 10-year survival rates with treatment similar to those of age- and sex-matched controls. Available data are consistent with improvements after treatment in some but not all hemochromatosis-related morbidities; however, none of these data are controlled, and studies do not generally ensure minimally valid measures of treatment response. Treatment may result in reduced insulin doses in insulin-dependent diabetics and elevated liver enzymes reductions. Liver biopsies before and after treatment suggest arresting of disease progression in most individuals, and a possible reduction in the severity of hepatic fibrosis, particularly in less severely affected patients. Symptoms such as extreme fatigue, abdominal pain, and lethargy improve in the majority of patients while arthralgia and impotence do not. Harms were not reported in any studies.

High-Risk Groups
(Key Question 3)

Family members of individuals with HH are at higher risk of having hemochromatosis, and family screening has been established as a standard of care based on HLA-typing studies of family members of probands.[80,81] We found two U.S. studies using *HFE*-genotyping to determine risk in probands and family members that support this practice. A high proportion of tested biologic relatives (14 to 49%) are also C282Y homozygotes. The highest proportion (49%) is seen in family members of probands identified clinically (as opposed to screening), although these data have limitations as discussed above.

Some have suggested a targeted approach to screening by identifying persons with signs or symptoms consistent with undiagnosed, early-stage hemochromatosis. Seven cross-sectional studies examined the proportion of patients with a range of hemochromatosis-consistent diseases or symptoms taken from various settings that were C282Y homozygotes. Findings may not be conclusive as many comparisons were based on fewer than 300 patients, which may be insufficient given the population prevalence of C282Y homozygotes (3 to 5 per 1000 Caucasians). Primary care patients selected for signs or symptoms had the same prevalence of C282Y homozygosity as did healthy controls; patients from rheumatology clinics and with a history of coronary or other cardiac disease showed no greater prevalence of C282Y homozygosity. A higher proportion of C282Y homozygotes could be identified by genotyping liver clinic patients pre-screened to have TS above 45% (7.7% C282Y/C282Y) or by targeting diabetics hospitalized for poor control or complications (5.5%) or patients who were referred to specialists for chronic fatigue and arthralgias (5.7%). While biochemical screening with TS and SF further enriched this patient pool, calculated specificity remained low (56 to 67%).

Overall Evidence

The quantity of evidence that met quality and relevance criteria for the focused key questions posed by this review was small, despite a very large published literature. After reviewing 1886 abstracts and 255 full-text articles, we located only 34 fair-to-good quality studies that were

relevant to some aspect of our three key questions on burden of disease, benefits of early treatment, and high-risk groups. Reasons for study exclusions are listed in our excluded studies tables. Some articles often cited to support screening and treatment benefits in this field did not meet minimal quality criteria for our review, as was true of often-cited data within the studies we could include. All of the reviewed evidence, including treatment studies, was observational, much of it representing the experience of a small number of relatively selective individuals, and much of it without data to allow comparisons with an unaffected population. The published research was often difficult to interpret consistently and accurately given incompleteness and extreme variability in reporting standards.

In reviewing this field, others have included a larger range of study designs, such as modeling expected genotyping frequency in older populations, autopsy studies, and other circumstantial approaches. Our focused key questions did not allow this type of evidence into our review, but it is unlikely that their inclusion would be of great use to the USPSTF, given its evidence hierarchy and requirement of at least fair quality evidence for making its recommendations.[50]

Limitations

The research literature for this field is extensive, but it is primarily retrospective, observational, and descriptive as opposed to analytical. A great deal was published prior to the availability of *HFE* genotyping for HH.

The articles we included required significant interpretation for data abstraction and synthesis. For individual articles, we typically reviewed all tables for possibly relevant data and checked text calculations. We made every effort to report data only on adult populations relevant to screening, which required careful reading and data dissection in studies that combined cases from many sources. We excluded studies with serious discrepancies or where outcomes could not be related back to a sample or population source we were addressing. Many articles required further hand calculations to extract data in the most comparable form to allow cross-study comparisons, and inconsistencies between tables and text in many articles complicated this process. The number of calculations and interpretation from descriptive data raise a concern about data errors. We screened a significant amount of literature to try and locate quality, relevant research in a relatively short time period; although we may have missed some articles or reported data that contained some information relevant to these key questions, none was brought to our attention by peer reviewers. Overall, the difficulties in understanding and interpreting this literature posed challenges to meet our usual standards of comprehensiveness and consistency.

We primarily focused on HH as the condition of interest for this screening review, and within that, on the most common associated *HFE* genotype in the U.S. (C282Y homozygosity), which accounts for 85 to 90% of cases in Caucasians. We did not examine other hereditary causes or the impact of *HFE* heterozygosity that may account for 3 to 5% of HH patients. Due to the focused nature of this review, studies that might have indirectly informed the data reviewed for each key question were excluded.

V. Conclusions

Based on this focused evidence review, research regarding screening for HH remains very limited. Despite the availability of new studies in response to calls for improved research,[18,48,82] not enough is known yet to allow a confident projection of the impact or benefit from wide-spread genotypic screening for HH. Data are beginning to be reported for targeted high-risk population screening approaches (e.g., high-risk identification followed by phenotypic screening followed by genotypic screening).

Recent studies suggest that disease expression or penetrance is less than 100% in C282Y homozygotes identified through some method of screening. How much less than 100% and for whom remains uncertain. In the next year or two, the HEIRS followup should provide information on short-term disease expression based on clinical exams of C282Y homozygotes, those with elevated iron measures at the time of screening, regardless of genotype, and a sample of controls. However, only self-reported disease expression data is available on all 99,000 genotyped and phenotyped primary care patients and follow-up beyond 1 to 2 years is not planned. If funding is provided, this study could be a rich resource of prospective information on disease development as well as observational data on treatment response in contemporarily diagnosed patients with clear disease definition. Without other data, such as might come from the HEIRS study, the literature on treatment remains quite small, consisting of dated case series in less than 500 patients (few of whom have HH documented by genotype). Controlled treatment trials will probably never be undertaken for ethical reasons, so higher quality observational treatment data would be very useful.

The limited literature on genotyping family members of C282Y/C282Y probands suggests a higher proportion of homozygous family members are C282Y homozygotes and that these are more likely to have phenotypic expression, compared with those identified from other screening approaches. This literature is also of limited quantity due to the relatively recent availability of *HFE* testing (1996), but there is a large body of HLA-based literature on which family screening of probands has been established. Research needs in this area remain high.[80]

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Table 1. Key questions.

1. Among those with a homozygous C282Y genotype, what is the risk of developing clinical hemochromatosis?

2. Does earlier therapeutic phlebotomy of individuals with primary iron overload due to HH reduce the morbidity and mortality compared with treatment after diagnosis in routine clinical care?

3. Are there groups at increased risk for developing HH that can be readily identified prior to genetic testing?

Table 2. Definitions.

Asymptomatic: with no or only general and vague symptoms like arthralgias, emotional distress, fatigue, abdominal pain and non-specific signs, such as elevated liver function tests.

Biochemical screening: measurement of transferrin saturation and/or serum ferritin to screen for primary iron overload.

Clinical hemochromatosis: diagnosed liver disease (fibrosis, cirrhosis, liver failure, hepatocellular carcinoma), cardiomyopathy, diabetes mellitus, or arthropathy in the presence of primary iron overload.

Elevated iron parameters: increased levels of body iron as reflected by elevations in serum transferrin saturation or serum ferritin.

Groups at increased risk for developing clinical hemochromatosis: includes asymptomatic individuals who can be identified by virtue of an associated factor or sign and who might be the focus of a targeted genetic screening program. Factors or signs could include age, sex, ethnicity, family history of iron overload or clinical hemochromatosis, increased liver function tests. Does not include those with existing disease (diabetes mellitus, cirrhosis, cardiomyopathy) where the effort is to detect hemochromatosis in order to treat the disease, as this is tertiary prevention.

Iron overload (IO): excess deposition of iron in liver diagnosed by liver biopsy or increased total body mobilizable iron diagnosed by quantitative phlebotomy. Criteria for diagnosis is liver biopsy specimen with hepatic iron index of 1.9, with or without fibrosis. In quantitative phlebotomy, iron overload (IO) represents the removal of > 4 grams of mobilizable iron to reach biochemical indicators of iron depletion. This corresponds approximately to > 90 µmol/g of hepatic iron or at least "moderate" iron overload (on scale of normal, mild IO, moderate IO, substantial IO and severe IO). "Iron overload" not meeting this standard may be considered possible or provisional primary iron overload.

Genotypic screening: detecting those with, or at risk for, developing iron overload or clinical hemochromatosis through genotyping the *HFE* gene to detect C282Y homozygosity.

Hemochromatosis (HC): term used variously in the literature, but here to mean manifest disease determined to be due to excess body iron, but not clearly fitting more precise etiologic definitions.

Hereditary hemochromatosis (HH): IO or clinical hemochromatosis due to C282Y homozygosity

Morbidity: organ damage that results in physical disability over and above that not seen in the absence of iron overload.

Phenotypic screening: detecting those with or at risk for developing clinical hemochromatosis through biochemical screening using serum ferritin and/or transferrin saturation.

Primary iron overload: due to an inherent, inherited defect in iron regulation.

Screening population: refers to a group of populations of individuals who are identified and tested in a manner that is not related to their symptoms – i.e. they are not *identified* through disease signs or symptoms. A screening population can be identified by their relationship to a proband, as long as their symptoms did not bring them to the attention of the researchers.

Targeted screening: screening those identified as high-risk for developing hemochromatosis as opposed to general population screening.

Therapeutic phlebotomy: the process of repeatedly drawing blood until iron parameters are within normal limits. Typically treatment schedule is one unit (500 mL) of blood, biweekly until serum ferritin < 20µg/l. Maintenance therapy of 3-4 units/yr is common.

Unselected hemochromatosis patients: those with primary hemochromatosis not clearly due to C282Y homozygosity but with secondary causes eliminated. A term created to describe a category of patients with genetic disease not clearly due to C282Y.

Wild type: in *HFE* genotyping, typically refers to individuals who do not have C282Y and/or H63D alleles, the alleles most commonly tested.

Table 3. Screening and diagnostic criteria for iron overload.

Term/Test	Males	Females
Screen-positive for Elevated Iron Parameters		
Transferrin Saturation **(TS)**[1-3]	>50%	>45%
Serum Ferritin **(SF)**[2,4]	>300µg/L	>200µg/L
Possible Iron Overload (PIO)		
(Repeat TS OR	> 50%	>45%
Repeat SF OR	>300µg/L	>200µg/L
Initial >TS and >SF) AND		
Clinical exam		
Provisional Primary Iron Overload (PPIO)[2]		
Repeat TS and SF both increased and not due to liver disease, inflammation, or secondary causes of IO		
Documented Iron Overload (IO)[2]		
Meets all the PPIO criteria and increased body iron stores by one or more of:		
• Hepatic iron concentration (from biopsy) **(HIC)**	• ≥ 90 µm/g, ≥ 5000 µg/g dry wt	
• Iron removed to reach iron depletion (phlebotomy)	• >4 g iron removed	
• Histology	• histology suggestive of HC <u>and</u> HII ≥ 1.9	
o Hepatic iron index **(HII)**		
• Hepatic iron staining	• 3+, 4+	

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Table 4. Longitudinal studies of disease development in C282Y homozygotes.

Study Ref	Population	Screening		Iron Overload		Morbidity		Quality
		Criteria*/sequence	Results	Criteria*	Results	Definitiion	Results	
Olynyk, 2004[52]	Retrospective examination of 3011 randomly selected subjects (ages 20-79 y) from Busselton cohort genotyped in 1998. Available data: 1981, 1994, & 1998	HFE genotype	16/3011 C282YY, 4 previously diagnosed and undergoing TP. These 4 excluded. Serum available on 10/12.	>90 µm/g	5/6 biopsied in 1998	Fibrosis (6 biopsied)	2/6	Good
						Cirrhosis	1 of 6 (cirrhotic patient drank > 6 alcohol drinks/day)	
		Elevated Iron Parameters TS > 45%	1981: 3 of 9 Median age: 30 y			DM	1 subject at 19 yr thought unrelated to HC	
			1994: 9 of 10 Median age: 43 y			Arthralgia	4/10	
			1998: 10 of 10 Median age: 47 y					
		SF > 300	1981: 5/10	Possible Iron Overload				
			1994: 5/10 4/5 were same	M: TS >50, SF > 300	4/4 (calculated)			
			1998: 6/10	F: TS >45, SF >200	2/6 (calculated)			

Table 4 (continued). Longitudinal studies of disease development in C282Y homozygotes.

		Screening		Iron Overload		Morbidity		
Study Ref	Population	Criteria*/sequence	Results	Criteria*	Results	Definitiion	Results	Quality
Andersen, 2004[53]	Retrospective cohort from Copenhagen Heart study 1976-2001 n = 9,174 White: > 99%47% (9,174/19,698) of original Copenhagen study population	*HFE* Genotype C282Y:C282Y	23/9174	Possible Iron Overload Criteria:		DM	1/23 (4%)	Fair
			20 still alive	M: TS >50, SF >300, and CE	5/7 (calculated)	Liver disease as defined by AST > 50 Alk phos >275 Coag < 70% Bili > 17	0/23 (0%)	
		TS > 50 in 2001	M: 5/7, F: 13/16					
		SF > 250 in 2001	M: 6/7	F: TS >45, SF >200, and CE	9/16 (calculated)			
		SF> 200 in 2001	F: 10/16			Clinical workups in 2001 for liver disease, hypogonadism, cardiomyopathy For arthralgias	0/23	
		Iron measure progression: (1976-2001)		Liver biopsies not done				
		Transferrin Saturation Female Mean age: 25 y	Mean: 50					
		Mean age: 85 y	Mean: 70				2/23	
		Male Mean age: 35 y	Mean: 70			Subclinical HC		
		Mean age: 80 y	Mean: 80				1/23	
		Serum Ferritin Female Mean age: 25 y	Mean: 120					
		Mean age: 85 y	Mean: 500					
		Male Mean age: 35 y	Mean: 800					
		Mean age: 80 y	Mean: 400					

*Criteria defined in Table 2.Selected C282YY-% positives in subset of C282Y homozygotes tested; All C282YY-% positives in all C282Y homozygotes.C282YY-C282Y/C282Y; NHANES-National Health and Nutrition Examination Survey ; TS-transferrin saturation; SF-serum ferritin; DM-diabetes mellitus; HR-; M-male; F-female; HIO-hepatic iron index; HIC-hepatic iron content; TP-therapeutic phlebotomy; Med-median; HC-hemochromatosis; AST-aspartate aminotransferase; Alk phos-alkaline phosphatase; Coag-coagulation tests; Bili-bilirubin.

Table 5. Cross-sectional genotypic screening studies (12 studies).

Population screened	Range of prevalence of C282Y homozygotes	Elevated transferrin saturation	Elevated serum ferritin	Iron overload	Diabetes	Elevated LFTs	Fibrosis/ Cirrhosis
Health clinics (n=50,434)[33,55,56]	3.7-5.7 per 1000	M: 80%	M: 70 - 76%	Selected C282YY: 13/54 24%	0 - 5.6%	ID	ID
Total C282YY studied: n=206 (156 not previously identified)	Avg: 4.1 per 1000 (calculated)	F: 40 - 41%	F: 54%	All C282YY: 13/102 12.8%			
Blood donors (n=16,842)[59-61]	1.5-3.1 per 1000	M: >50 60 - 80%	M: 40-80%	ID	0%	ID	ID
Total C282YY studied: n=42	Avg: 2.5/1000 (calculated)	F: >45 27-67%	F: 0 - 9%				
General population (n=3011)[62]	5.3 per 1000	>45 x 2	58% - (excludes those treated)	Selected C282YY: 4/7 (57.1%)	ID	ID	Cirrhosis or Fibrosis: Selected C282YY: 3/7 (43%) All C282YY: 3/12 (25%)
Total C282YY studied: n=16 (12 not previously identified)		93.8%		All C282YY: 4/12 (33%)			Fibrosis: Selected C282YY: 2/7 All C282YY: 2/12
							Cirrhosis: Selected C282YY: 1/7 All C282YY: 1/12
Voter rolls and employee screening (n=14,326)[63-66]	4.0-4.7 per 1000	>50-65	61.5%	Selected C282YY: 6/8 (75%) All C282YY: 6/13 (46%)	ID	ID	Fibrosis: Selected C282YY: 2/9(22%) All C282YY: 2/60 (3%)
Total C282YY studied: n=64 (60 not previously identified)	Avg: 4.5 per 1000 (calculated)	65-84.6%					
Family of probands(n=150)[67]	161/1000	87.5%	96%	ID	16% (4/25)	ID	Cirrhosis = 2/25 (8%)
Total C282YY studied: n=25							

LFT-liver function test; F-female; M-male; YY-C282Y/C282Y; ID-insufficient data; Avg-average; C282YY-C282Y/C282Y; Selected C282YY-% positives in only those tested; All C282YY: % positives in all C282YY.
*Patient consumed > 60 g of alcohol/day

Table 6. Summary estimates for genotypic screening yields from longitudinal and cross-sectional studies.

LONGITUDINAL STUDIES	Prevalence of C282Y homozygotes	Elevated TS and SF in homozygotes	Iron overload due to HH	Diabetes	Elevated Liver function tests	Fibrosis or cirrhosis
General population (2 studies)						
Anderson, 2004[53]	2.5/1000	M: 5/7 F: 9/16 (both combined)	Selected C282YY:ND All C282YY: ND	All C282YY: 1/23 4.4%	ND	ND
Olynyk 2004[52]	4/1000	M: 4/4 F: 2/6 (both tests combined)	Selected C282YY: 5/6 All C282YY: 5/10	ND	ND	Selected C282YY: 3/6 (1also drank) All C282YY: 3/10 (30%)

CROSS-SECTIONAL STUDIES	Prevalence of C282Y homozygotes	Elevated TS in C282Y homozygotes	Elevated SF in C282Y homozygotes	Iron overload due to HH	Diabetes	Elevated Liver function tests	Fibrosis or cirrhosis
General Population (7 studies) (n=67,771)[33,56,62-66] Total C282YY studied: n=282	4.2/1000	M: 75-94% F: 40-94%	M: 58-76% F: 54-58%	*Selected C282YY: 26/69 = 38% *All C282YY: 30/127 = 24%	All C282YY: max-5.6% min-0%	All C282YY: max-ND min-ND	**Cirrhosis or Fibrosis: Selected C282YY: 5/16 (31%) All C282YY: 5/72 (6.9%) **Fibrosis: Selected C282YY: 4/16 (25%) All C282YY: 4/72 (6%) **Cirrhosis: Selected C282YY: 1/16 (6%) All C282YY: 1/72 (1.4%)
Family History (1 study) (n=150)[67] Total C282YY studied: n=25	161/1000 25/150	M & F: 87.5%	M & F: 96% 23/25 = 92%	All C282YY: ND	All C282YY: 16%	All C282YY: ND	Selected C282YY: ND All C282YY: 2/25 8%

Only included those ≥ 10.

* Data from Beutler, Burt, Distante, McDonnell, Olynyk.

** Data from Burt, Delatycki, Distante, Olynyk.

C282YY-C282Y/C282Y; F-female; M-male; ND- no data reported or not acceptable; Selected C282YY-% positives in only those tested; All C282YY-% positives in all C282YY; max-maximum; min-minimum

Table 7. Prevalence of C282Y homozygotes in phenotypically screened high-risk populations.

Study	Screening criteria		% Positive (n)		# C282Y/C282Y in screen positive patients		% C282Y/C282Y in screen positive patients	
	TS, %	SF, µg/L	TS	SF	TS	SF	TS	SF
Poullis, 2003[73]								
Liver clinic (n=667)	> 45	> 300	23% (156)		11/156		7.1	
Cadet, 2003[75]								
Rheumatology (n=221)	> 45	> 300	4% (9)	4% (9)	ND	ND	ND	ND
Endocrinology (n=121)	> 40	> 300	88% (106)	46% (56)	7/106	6/56	6.6%	10.7%
Specialty setting: fatigue/arthritis (n=227)	> 40	> 300	31% (70)	33% (75)	13/70	13/75	17.3%	18.6%
Health appraisal-healthy volunteers (n=991)	> 40	> 300	30% (293)	6% (57)	2/293	0/57	0	0.6%
Swinkels, 2002[77]								
Chronic fatigue syndrome (n=88)	F: > 40 M: > 45	F pre-menopause: > 80 F post-menopause: ≥ 190 M: > 280	7% (6)	2% (2)	ND	ND	ND	ND

TS-transferrin saturation; SF-serum ferritin; ND-not determined-denominator too small; F-female; M-male

Figure 1. Secular trends in hemochromatosis associated morbidity. (Niederau, et al Long term survival in patients with hereditary hemochromatosis. Gast 110(4):1107-19, 1996)[26]
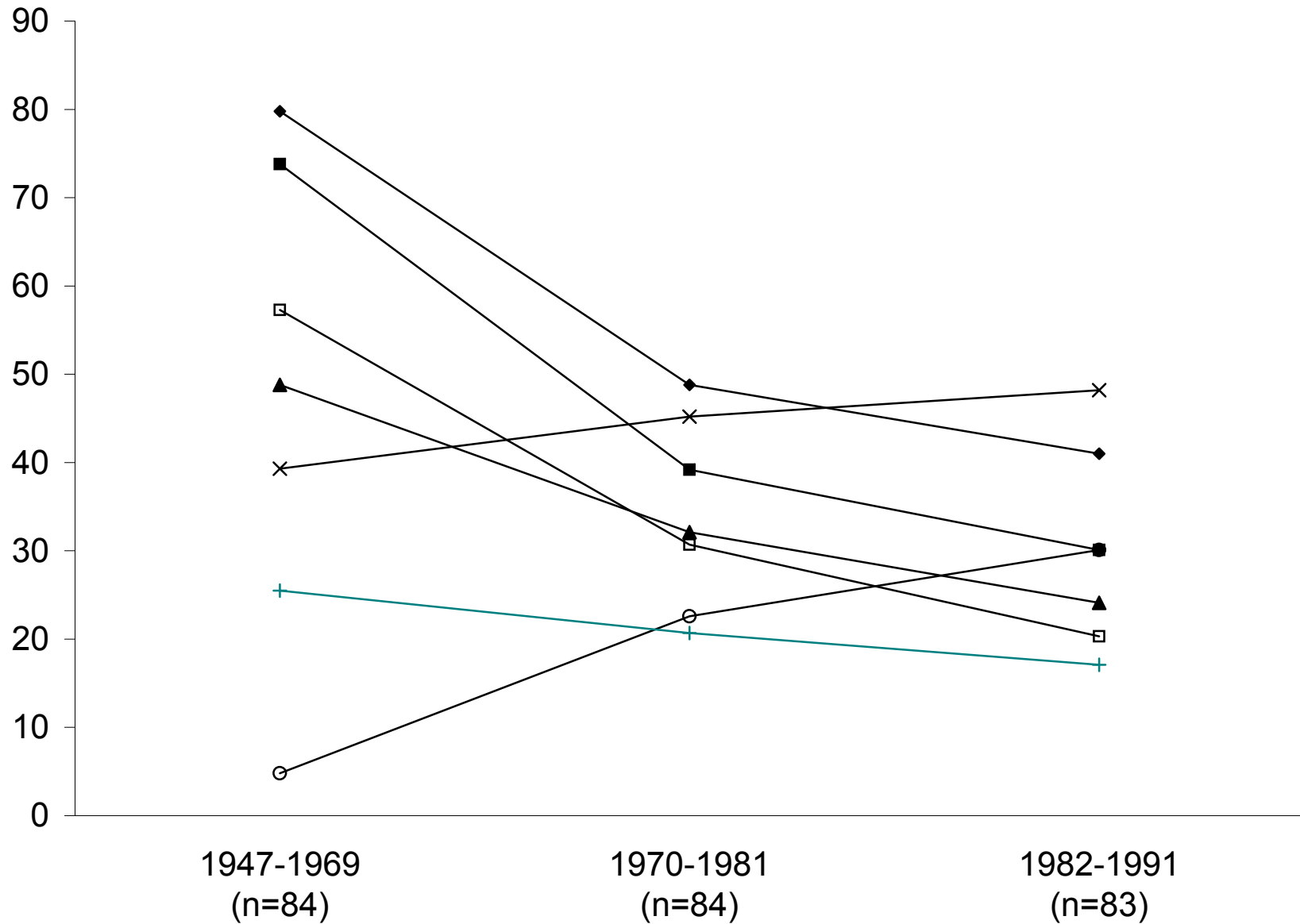
Liver cirrhosis(%) ♦, Diabetes mellitus (%) ■, Electrocardiographic changes (%) ▲, Arthralgia (%) x, Loss of potency (%) □, Diagnosis of asymptomatic patients (%) ○, Mobilizable iron (g) +

34

Appendix A Figure 1. Search results and article flow by key question.
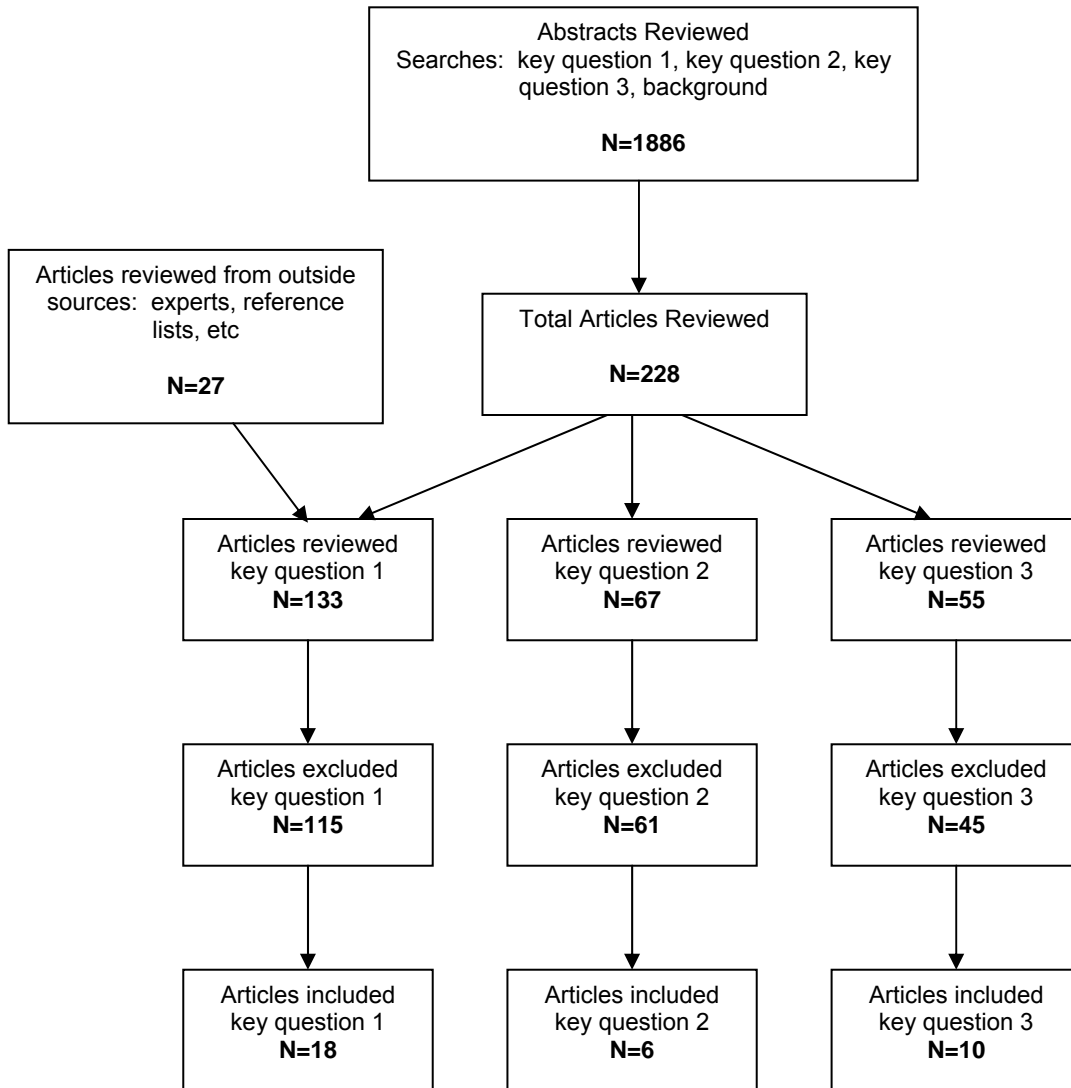
Appendix A Figure 2. Abstract and article review process for hemochromatosis.

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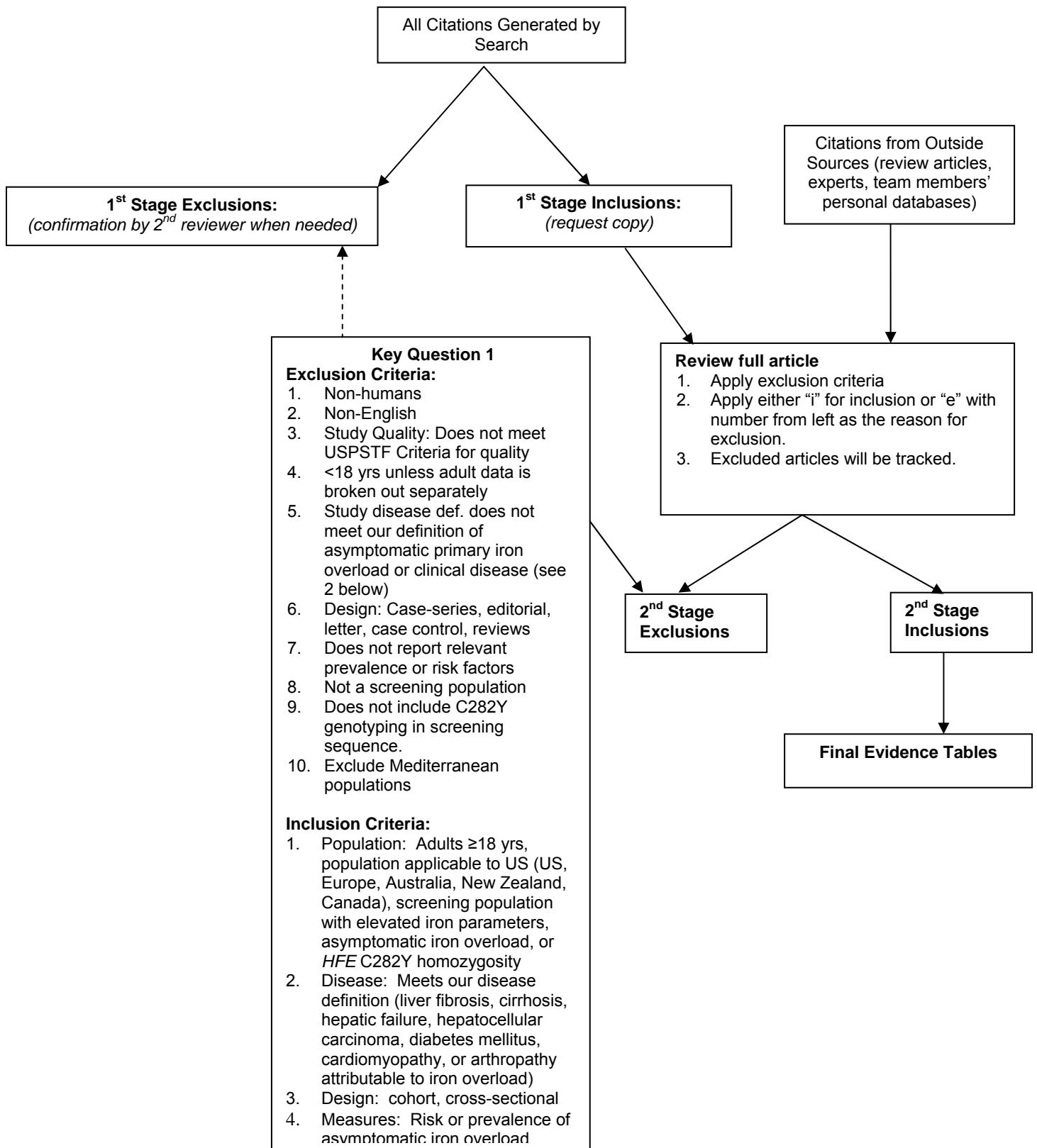
1st Stage Exclusions:
(confirmation by 2nd reviewer when needed)

1st Stage Inclusions:
(request copy)

Citations from Outside Sources (review articles, experts, team members' personal databases)

Key Question 1
Exclusion Criteria:
1. Non-humans
2. Non-English
3. Study Quality: Does not meet USPSTF Criteria for quality
4. <18 yrs unless adult data is broken out separately
5. Study disease def. does not meet our definition of asymptomatic primary iron overload or clinical disease (see 2 below)
6. Design: Case-series, editorial, letter, case control, reviews
7. Does not report relevant prevalence or risk factors
8. Not a screening population
9. Does not include C282Y genotyping in screening sequence.
10. Exclude Mediterranean populations

Inclusion Criteria:
1. Population: Adults ≥18 yrs, population applicable to US (US, Europe, Australia, New Zealand, Canada), screening population with elevated iron parameters, asymptomatic iron overload, or *HFE* C282Y homozygosity
2. Disease: Meets our disease definition (liver fibrosis, cirrhosis, hepatic failure, hepatocellular carcinoma, diabetes mellitus, cardiomyopathy, or arthropathy attributable to iron overload)
3. Design: cohort, cross-sectional
4. Measures: Risk or prevalence of asymptomatic iron overload

Review full article
1. Apply exclusion criteria
2. Apply either "i" for inclusion or "e" with number from left as the reason for exclusion.
3. Excluded articles will be tracked.

2nd Stage Exclusions

2nd Stage Inclusions

Final Evidence Tables

Appendix A Figure 2, continued. Abstract and article review process for hemochromatosis.

Key Questions 2	**Key Question 3**
Exclusion Criteria:	**Exclusion Criteria:**
1. Non-humans	1. Non-humans
2. Non-English	2. Non-English
3. Study Quality: Does not meet USPSTF Criteria for quality	3. Study Quality: Does not meet USPSTF Criteria for quality
4. <18 yrs unless adult data is broken out separately	4. <18 yrs unless adult data is broken out separately
5. Study disease def. does not meet our definition of disease (see 2 below)	5. Study disease def does not meet our definition of disease (see 2 below)
6. Design: Case-studies, editorial, letter or case-series of < 20 patients, reviews	6. Design: Case-series, editorial, letters, reviews
7. Does not report relevant outcomes	7. Does not report relevant prevalence or risk measures
8. Not phlebotomy treatment	8. Does not include original data
9. Exclude Mediterranean populations	9. Not the correct population
	10. Exclude Mediterranean populations
	11. No HFE testing
Inclusion Criteria:	**Inclusion Criteria:**
1. Population: Adults ≥18 yrs, population applicable to US (US, Europe, Australia, New Zealand, Canada), primary Fe overload	1. Population: Adults ≥18 yrs, population applicable to US (US, Europe, Australia, New Zealand, Canada)
2. Disease: Meets our disease definition (liver fibrosis, cirrhosis, hepatic failure, hepatocellular carcinoma, diabetes mellitus, cardiomyopathy, or arthropathy attributable to iron overload)	2. Disease: Meets our disease definition (liver fibrosis, cirrhosis, hepatic failure, hepatocellular carcinoma, diabetes mellitus, cardiomyopathy, or arthropathy attributable to iron overload)
3. Outcomes: Incidence, severity or progression of clinical hemochromatosis or iron measures, non-specific symptoms	3. Design: cohort, case control, cross-sectional
	4. Prevalence or incidence of hemochromatosis or risk of developing hemochromatosis

Appendix A. Search strategies.

Databases: Medline, DARE, Cochrane Database of Systematic Reviews, Cochrane Central Register of
Controlled Trials

Dates searched: 1966-February 2005

Key Question 1

1	HEMOCHROMATOSIS/
2	hemochromatosis.ti,ab.
3	haemochromatosis.ti,ab.
4	Iron Overload/
5	iron overload.ti,ab.
6	c282y.ti,ab.
7	1 or 2 or 3 or 4 or 5 or 6
8	cohort studies/ or longitudinal studies/ or follow-up studies/ or prospective studies/
9	follow-up stud$.ti,ab.
10	cohort stud$.ti,ab.
11	longitudinal$.ti,ab.
12	prospective$.ti,ab.
13	INCIDENCE/
14	incidence.ti,ab.
15	predict$.ti,ab,hw.
16	natural history.ti,ab.
17	penetrance/
18	penetran$.ti,ab.
19	clinical expression$.ti,ab.
20	clinical presentation$.ti,ab.
21	clinical consequence$.ti,ab.
22	clinical feature$.ti,ab.
23	clinical manifestation$.ti,ab.
24	8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
25	7 and 24
26	limit 25 to (humans and english language)
27	limit 26 to "all child (0 to 18 years)"
28	limit 27 to "all adult (19 plus years)"
29	27 not 28
30	26 not 29
31	(editorial or letter or news).pt.
32	30 not 31

Key Question 2

1	HEMOCHROMATOSIS/
2	hemochromatosis.ti,ab.)
3	haemochromatosis.ti,ab.
4	Iron Overload/
5	iron overload.ti,ab.
6	1 or 2 or 3 or 4 or 5
7	BLOODLETTING/
8	blood lett$.ti,ab.)
9	PHLEBOTOMY/
10	phlebotom$.ti,ab.
11	venesect$.ti,ab.
12	7 or 8 or 9 or 10 or 11
13	6 and 12
14	Hemochromatosis/th [Therapy]
15	Iron Overload/th [Therapy]
16	13 or 14 or 15
17	limit 16 to (humans and english language)
18	limit 17 to "all child (0 to 18 years)"
19	limit 18 to "all adult (19 plus years)"
20	18 not 19

21	17 not 20
22	(editorial or letter or news).pt.
23	21 not 22

Key Question 3

1	HEMOCHROMATOSIS/
2	hemochromatosis.ti,ab.
3	haemochromatosis.ti,ab.
4	1 or 2 or 3
5	family/ or nuclear family/ or parents/ or fathers/ or mothers/ or siblings/
6	(family or families).ti,ab.
7	(relative or relatives).ti,ab.
8	sibling$.ti,ab.
9	(mother$ or father$).ti,ab.
10	parent$.ti,ab.
11	5 or 6 or 7 or 8 or 9 or 10
12	screen$.ti,ab,hw.
13	diagnos$.ti,ab,hw.
14	di.fs.
15	12 or 13 or 14
16	4 and 11 and 15
17	target$.ti,ab.
18	4 and 15 and 17
19	Risk Factors/
20	risk factor$.ti,ab.
21	increased risk$.ti,ab.
22	high risk.ti,ab.
23	prognostic factor$.ti,ab.
24	19 or 20 or 21 or 22 or 23
25	4 and 24
26	cascad$.ti,ab.
27	4 and 26
28	Liver Function Tests/
29	liver function.ti,ab.
30	(abnormal$ adj3 liver).ti,ab.
31	(increased adj3 liver).ti,ab.
32	(elevate$ adj3 liver).ti,ab.
33	28 or 29 or 30 or 31 or 32
34	4 and 33
35	16 or 18 or 25 or 27 or 34
36	limit 35 to english language
37	limit 36 to humans
38	limit 37 to "all child (0 to 18 years)"
39	limit 38 to "all adult (19 plus years)"
40	38 not 39
41	37 not 40
42	(editorial or letter or news).pt.
43	41 not 42

Background

1	hemochromatosis)
2	hemochromatosis.ti,ab.
3	haemochromatosis.ti,ab.
4	1 or 2 or 3
5	PREVALENCE/
6	prevalen$.ti,ab.
7	5 or 6
8	4 and 7
9	HEMOCHROMATOSIS/ep [Epidemiology]
10	mo.fs.

11	"Cause of Death"/
12	Survival Rate/
13	Life Expectancy/
14	mortality.ti,ab.
15	10 or 11 or 12 or 13 or 14
16	4 and 15
17	8 or 9 or 16
18	limit 17 to english language
19	limit 18 to humans
20	limit 19 to "all child (0 to 18 years)"
21	limit 20 to "all adult (19 plus years)"
22	20 not 21
23	19 not 22
24	(letter or news or editorial).pt.
25	23 not 24

Appendix B. USPSTF Hierarchy of research design and quality rating criteria. [50]

Hierarchy of Research Design

I	Properly conducted randomized controlled trial (RCT)
II-1:	Well-designed controlled trial without randomization
II-2:	Well-designed cohort or case-control analytic study
II-3:	Multiple time series with or without the intervention; dramatic results from uncontrolled experiments
III:	Opinions of respected authorities, based on clinical experience; descriptive studies or case reports; reports of expert committees

Design-Specific Criteria

Systematic Reviews

Criteria:
- Comprehensiveness of sources considered/search strategy used
- Standard appraisal of included studies
- Validity of conclusions
- Recency and relevance are especially important for systematic reviews

Case-Control Studies

Criteria:
- Accurate ascertainment of cases
- Nonbiased selection of cases/controls with exclusion criteria applied equally to both
- Response rate
- Diagnostic testing procedures applied equally to each group
- Measurement of exposure accurate and applied equally to each group
- Appropriate attention to potential confounding variables

Randomized Controlled Trials and Cohort Studies

Criteria:
- Initial assembly of comparable groups
  - -for RCTs: adequate randomization, including first concealment and whether potential confounders were distributed equally among groups.
  - -for cohort studies: consideration of potential confounders with either restriction or measurement for adjustment in the analysis; consideration of inception cohorts
- Maintenance of comparable groups (includes attrition, crossovers, adherence, contamination)
- Important differential loss to follow-up or overall high loss to follow-up
- Measurements: equal, reliable, and valid (includes masking of outcome assessment)
- Clear definition of the interventions
- All important outcomes considered

Diagnostic Accuracy Studies

Criteria:
- Screening test relevant, available for primary care, adequately described
- Study uses a credible reference standard, performed regardless of test results
- Reference standard interpreted independently of screening test
- Handles indeterminate result in a reasonable manner
- Spectrum of patients included in study
- Sample size
- Administration of reliable screening test

Appendix C Table 1. Genotype screening studies in various populations.

Study	Population	C282Y:C282Y frequency	TF saturation* (initial test unless stated)	Serum ferritin*	Iron overload	Diabetes	Elevated liver enzymes	Hepatic fibrosis or cirrhosis
Health clinics								
Beutler 2002a[33]; Beutler 2002b[55], Beutler 2000[54], Waalen 2002[58]	KP San Diego n = 41,038 Mean age: 57 y Non-Hispanic Caucasian: 77%	152/41,038 3.7/1000	>50: 75% M 40% F Elev overall: 57% After excluding frequent blood donors: 76% M, 41% F	>250 M: 76% >200 F: 54% Elev overall: 65% After excluding frequent blood donors: 77% M, 56% F	NR	C282YY: 5.6% Non-C282YY: 8.4%	AST > 40 C282YY: 8.2% Non-C282YY: 3.8%	NR
	140 C282YY from KP San Diego screening study				102 eligible (not prev treated) 54 completed treatment 13/54 (24%) had >5 g iron removed			
Deugnier 2002[56]	Brittany, France n = 9396 35.8% male (deliberately weighted to include younger males)	54/9396 5.7/1000	>55 M: 80% >50 F: 41% Elev overall: 48%	>280 M: 70% >130 F: 33% Elev overall: 40%	NR	M C282YY: 0% M non-C282YY: 0.8% F C282YY: 2.3% F non-C282YY: 0.9%	ALT>70 M C282YY: 10% M Non-C282YY: 5% M ALT > 35 F C282YY: 5% F Non-C282YY: 5% F	
Phatak 2002**[57]	Rochester NY n = 4865 randomly selected Mean age: 52 y 42% male 66% Caucasian	12 of 4865 2.5/1000	>45: 75% M,100% F >50: 75% M, 88% F >45 Overall: 92% >50 Overall: 83%	>200: 100% M, 50% F, Overall 67% >300: 50% M, 37.5% F, Overall 41%	4/5 receiving TP had > 4g removed. 4/4 biopsied had HIO (criteria not given) At least 4/12 had HIO and possibly 8/12-not sure	0/12 = 0%	NR	Cirrhosis: 0% of 4 biopsied

Appendix C Table 1 (continued). Genotype screening studies in various populations

Study	Population	C282Y:C282Y frequency	TF saturation* (initial test unless stated)	Serum ferritin*	Iron overload	Diabetes	Elevated liver enzymes	Hepatic fibrosis or cirrhosis
Blood donor populations								
Adams 2000[59]	Canadian blood donors n = 5211 56.7% male 96% full European ancestry	16/5211 3.1/1000	> 50 X 2 M: 3/5 (60%) > 45 X 2 F: 3/11 (27%) Combined: 6/16 (38%)	>300 M: 40% >200 F: 9% Overall: 18.8%	NR	0/16 or 0%	Elev AST (ND): 0/16 or 0% Elev ALT (ND): 0/16 or 0%	NR
Chambers 2003[61]	Male English blood donors who donated < 4 units previously n = 6261 Mean age = 39 y	18/6261 2.8/1000	> 50: 78%	>250: 56% >500: 11%	NR	NR	NR	NR
Sanchez 2003[60]	Barcelona, Spain n = 5370 blood donors 64% Male Mean age: 28 y	8 of 5370 1.5/1000	>50 on 1st: 80% M, 67% F	> 300: 80% M, 0% F	> 4 g removed: 2/3 (67%) of those with phlebotomy 25% of C282YY	NR	NR	NR

Appendix C Table 1 (continued). Genotype screening studies in various populations

Study	Population	C282Y:C282Y frequency	TF saturation* (initial test unless stated)	Serum ferritin*	Iron overload	Diabetes	Elevated liver enzymes	Hepatic fibrosis or cirrhosis
Population screening								
Olynyk 1999[62]	Busselton Australia n = 3011 randomly selected 50% Male Predom white Age range: 20 - 79 y	16/3011 5.3/1000 4/16 prev diagnosed 12 new C282YY (5.3/1000)	>45: 93.8% 2nd > 45: 93.8%	>300: 50% >300 untreated: 58.3%	Liver biopsy: 58% 7/12 HII>1.9: 4/7 (57.1%) of those biopsied 33% of C282YY HIC> 20 µmol/g dry: 100% of those biopsied (7/7) 58% C282YY	NR	NR	Fibrosis: 29%of biopsied (2/7) Cirrhosis: 14% of biopsied (1/7)(also had history alcohol >60 g/day) No Controls
Voter Rolls								
Burt, 1998[63]	1064 voters New Zealand Mean age: 50 y 39.8% Male	5/1064 4.7/1000	> 55: 100%	2nd SF >300 M: 100% >160 F: 50% Overall elev: 60%	Liver biopsy: 60% HII>1.9: 3/3 (100%) selected C282YY 3/5 (60%) all C282YY Grade 3-4: 33% of those biopsied 20% of all C282YY	NR	NR	NR

Study	Population	C282Y:C282Y frequency	TF saturation* (initial test unless stated)	Serum ferritin*	Iron overload	Diabetes	Elevated liver enzymes	Hepatic fibrosis or cirrhosis
Employment screening								
Distante 1999[64]	505 hospital employees in Oslo Norway 79% Female Mean age: 38 y	2/505 4/1000	> 50: 100%	> 200: 100%	TP on 50%: 5.2 g removed 1/1 IO by TP HIC: 47µmol Biopsy 0/1 IO: 50% selected C282YY Total IO = 100%	NR	NR	NR
McDonnell 1999[65]	1450 HMO employees in Springfield,MO 83% women 98% white Mean age:41 y	6/1450 4.1/1000	>50 x 2 F >60 x 2 M Overall elev: 67% of C282YY	>95%ile for age and sex: 50% C282YY	HII = 2.2: 1/1 by biopsy 1/2 by TP 2/3 (67%) selected C282YY 2/6 (33%) all C282YY	NR	NR	Fibrosis: 0/1 (0%) Cirrhosis: 0/1 (0%)
Delatycki 2005[66]	11,307 workplace employees in Australia 47% male 63% N. European	51/11,307 4 previous diagnosed 4.5/1000 47 new C282YY	Criteria for elevation not given, 65% were "elevated"	NR	6 recommended 4 biopsied	NR	NR	Fibrosis 2/4 biopsied 50% selected C282YY, 2/47 (4.3%) all C282YY

Appendix C Table 1 (continued). Genotype screening studies in various populations

Study	Population	C282Y:C282Y frequency	TF saturation* (initial test unless stated)	Serum ferritin*	Iron overload	Diabetes	Elevated liver enzymes	Hepatic fibrosis or cirrhosis
Family studies								
Barton 1999[67]	150 relatives of 61 probands in Alabama 100% Caucasian 52% female Mean age: 46 y 1 pt <18, was C282YY	25/149 161/1000	>50 F x 2 >60 M x 2 Overall: 87.5%	>300 M >200 F Overall 96% elev	NR	16%	NR	2/25 (8%)
Adams 1998**[68]	51 C282YY relatives of probands 70% male Mean age M: 52 y Mean age F: 57 y	Not able to calculate	>55: 33/38 (87%) M 11/13 (92%) F	>300 M: 34/38 (89%) >200 F: 12/13 (92%)	>5 g: 9/23 (39%) M 3/5 (60%) F All : 12/51(24%) HII > 1.9: 19/22 (86%) M 6/7 (86%) F All : 25/51 (49%)			

* In homozygotes

**Data not included in summary estimates table

Elev-elevated; F-female; M-male; C282YY-C282Y/C282Y; AST-aspartate aminotransferase; ND-not determined; HII-hepatic iron index; HIC-hepatic iron content; SF-serum ferritin; TP-therapeutic phlebotomy; IO-iron overload

Appendix C Table 2. Studies excluded from key question 1.

Study Citation	Reason for exclusion
Iron overload disorders among Hispanics--San Diego, California, 1995. *MMWR - Morbidity & Mortality Weekly Report 45(45):991-3,* 1996.	Study disease definition does not meet our definition of asymptomatic primary iron overload or clinical disease.
A simple genetic test identifies 90% of UK patients with haemochromatosis. The UK Haemochromatosis Consortium. *Gut 41(6):841-4,* 1997.	Not a screening population
Adams PC, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, Dawkins FW, Acton RT, Harris EL, Gordeuk VR, Leiendecker-Foster C, Speechley M, Snively BM, Holup JL, Thomson E, Sholinsky P, and Hemochromatosis and Iron Overload Screening (HEIRS) Study Research Investigators. Hemochromatosis and iron-overload screening in a racially diverse population. *New England Journal of Medicine.352(17):1769-78*, 2005.	Does not report relevant outcomes
Adams PC. Is there a threshold of hepatic iron concentration that leads to cirrhosis in C282Y hemochromatosis? *American Journal of Gastroenterology 96(2):567-9,* 2001.	Not a screening population
Adams PC, Deugnier Y, Moirand R, Brissot P. The relationship between iron overload, clinical symptoms, and age in 410 patients with genetic hemochromatosis. *Hepatology.25(1):162-6,* 1997.	Not a screening population
Adams PC, Gregor JC, Kertesz AE, Valberg LS. Screening blood donors for hereditary hemochromatosis: decision analysis model based on a 30-year database. *Gastroenterology.109(1):177-88*, 1995.	Does not contain primary data
Adams PC, Kertesz AE, Valberg LS. Clinical presentation of hemochromatosis: a changing scene. *American Journal of Medicine 90(4):445 -9,* 1991.	Not a screening population
Adams PC, Speechley M, Kertesz AE. Long-term survival analysis in hereditary hemochromatosis. *Gastroenterology 101(2):368-72,* 1991.	Not a screening population
Adams PC. Hepatic iron in hemochromatosis. *Digestive Diseases & Sciences.35(6):690-2*, 1990.	Includes data from patients < 18 yrs
Ammann RW, Muller E, Bansky J, Schuler G, Hacki WH. High incidence of extrahepatic carcinomas in idiopathic hemochromatosis. *Scandinavian Journal of Gastroenterology 15(6):733-6,* 1980.	Not a screening population
Asberg A, Hveem K, Kruger O, Bjerve KS. Persons with screening-detected haemochromatosis: as healthy as the general population? *Scandinavian Journal of Gastroenterology.37(6):719-24*, 2002.	Study disease definition does not meet our definition of asymptomatic primary iron overload or clinical disease.
Asberg A, Hveem K, Thorstensen K, Ellekjter E, Kannelonning K, Fjosne U, Halvorsen TB, Smethurst HB, Sagen E, Bjerve KS. Screening for hemochromatosis: high prevalence and low morbidity in an unselected population of 65,238 persons. *Scandinavian Journal of Gastroenterology 36 (10):1108-1115*, 2001.	Does not include C282Y genotyping in screening sequence
Askari AD, Muir WA, Rosner IA, Moskowitz RW, McLaren GD, Braun WE. Arthritis of hemochromatosis. Clinical spectrum, relation to histocompatibility antigens, and effectiveness of early phlebotomy. *American Journal of Medicine 75(6):957 -65,* 1983.	Not a screening population
Assy N, Adams PC. Predictive value of family history in diagnosis of hereditary hemochromatosis. *Digestive Diseases & Sciences 42(6):1312 -5,* 1997.	Study design

Study Citation	Reason for exclusion
Bacon BR, Sadiq SA. Hereditary hemochromatosis: presentation and diagnosis in the 1990s. *American Journal of Gastroenterology 92(5):784 -9,* 1997.	Not a screening population
Baer DM, Simons JL, Staples RL, Rumore GJ, Morton CJ. Hemochromatosis screening in asymptomatic ambulatory men 30 years of age and older. *American Journal of Medicine 98 (5):464-468*, 1995.	Does not include C282Y genotyping in screening sequence
Balan V, Baldus W, Fairbanks V, Michels V, Burritt M, Klee G. Screening for hemochromatosis: a cost-effectiveness study based on 12,258 patients. *Gastroenterology 107 (2):453-459*, 1994.	Does not include C282Y genotyping in screening sequence
Barosi G, Salvaneschi L, Grasso M, Martinetti M, Marchetti M, Bodini U et al. High prevalence of a screening-detected, HFE-unrelated, mild idiopathic iron overload in Northern Italy. *Haematologica 87(5):472-8,* 2002.	Does not report relevant outcomes
Barton JC, Cheatwood SM, Key TJ, Acton RT. Hemochromatosis detection in a health screening program at an Alabama forest products mill. *Journal of Occupational & Environmental Medicine.44(8):745-51*, 2002.	Does not report relevant outcomes
Barton JC, Barton NH, Alford TJ. Diagnosis of hemochromatosis probands in a community hospital. *American Journal of Medicine 103(6):498 -503,* 1997.	Not a screening population
Barton CJ, Shih WW, Sawada-Hirai R, Acton RT, Harmon L, Rivers C, Rothenberg BE. Genetic and clinical description of hemochromatosis probands and heterozygotes: evidence that multiple genes linked to the major histocompatibility complex are responsible for hemochromatosis. *Blood Cells Molecules & Diseases.23(1):135-45; discussion 145a.-b*, 1997.	Not a screening population
Bassett ML, Halliday JW, Ferris RA, Powell LW. Diagnosis of hemochromatosis in young subjects: predictive accuracy of biochemical screening tests. *Gastroenterology.87(3):628.-33*, 1984.	< 18 yrs included
Bassett ML, Halliday JW, Powell LW. Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis. *Hepatology.6(1):24-9,-Feb*, 1986.	Does not report relevant outcomes
Bell H, Thordal C, Raknerud N, Hansen T, Bosnes V, Halvorsen R, Heier HE, Try K, Leivestad T, Thomassen Y. Prevalence of hemochromatosis among first-time and repeat blood donors in Norway. *Journal of Hepatology 26 (2):272-279*, 1997.	Does not include C282Y genotyping in screening sequence
Bell H, Berg JP, Undlien DE, Distante S, Raknerud N, Heier HE , Try K, Thomassen Y, Haug E, Raha-Chowdhury R, Thorsby E. The clinical expression of hemochromatosis in Oslo, Norway. Excessive oral iron intake may lead to secondary hemochromatosis even in HFE C282Y mutation negative subjects. *Scandinavian Journal of Gastroenterology.35(12):1301-7*, 2000.	Not a screening population
Borwein ST, Ghent CN,. Flanagan PR, Chamberlain MJ, Valberg LS. Genetic and phenotypic expression of hemochromatosis in Canadians. *Clinical & Investigative Medicine - Medecine Clinique et Experimentale.6(3):171-9,* 1983.	Does not report relevant outcomes
Bradbear RA, Bain C, Siskind V, Schofield FD, Webb S, Axelsen EM et al. Cohort study of internal malignancy in genetic hemochromatosis and other chronic nonalcoholic liver diseases. *Journal of the National Cancer Institute 75(1):81-4,* 1985.	Not a screening population

Appendix C Table 2 (continued). Studies excluded from key question 1.

Study Citation	Reason for exclusion
Bradley LA, Haddow JE, Palomaki GE. Population screening for haemochromatosis: a unifying analysis of published intervention trials. *Journal of Medical Screening.3(4):178-84,* 1996.	Review article
Bulaj ZJ, Ajioka RS, Phillips JD, LaSalle BA, Jorde LB, Griffen LM, Edwards CQ, Kushner JP. Disease-related conditions in relatives of patients with hemochromatosis. *New England Journal of Medicine.343(21):1529-35*, 2000.	Quality
Buysschaert M, Paris I, Selvais P, Hermans MP. Clinical aspects of diabetes secondary to idiopathic haemochromatosis in French-speaking Belgium. *Diabetes & Metabolism 23(4):308-13,* 1997.	Case series
Cadet E, Capron D, Gallet M, Omanga-Leke ML, Boutignon H, Julier C, Robson KJ, Rochette J. Reverse cascade screening of newborns for hereditary haemochromatosis: a model for other late onset diseases? *Journal of Medical Genetics.*42(5):390-5, 2005.	Includes data from patients < 18 yrs Cannot separate C282Y homozygotes from C282Y heterozygotes
Cartwright GE, Edwards CQ, Kravitz K, Skolnick M, Amos DB, Johnson A et al. Hereditary hemochromatosis. Phenotypic expression of the disease. *New England Journal of Medicine 301(4):175-9,* 1979.	Does not report relevant outcomes
Cecchetti G, Binda A, Piperno A, Nador F, Fargion S, Fiorelli G. Cardiac alterations in 36 consecutive patients with idiopathic haemochromatosis: polygraphic and echocardiographic evaluation. *European Heart Journal 12(2):224-30,* 1991.	Not a screening population
Cogswell ME, Gallagher ML, Steinberg KK, Caudill PhD SP, Looker AC, Bowman BA et al. HFE genotype and transferrin saturation in the United States. *Genetics in Medicine 5(4):304-10,* 2003;-Aug.	Study disease definition does not meet our definition of asymptomatic primary iron overload or clinical disease.
Crawford DH, Jazwinska EC, Cullen LM, Powell LW. Expression of HLA-linked hemochromatosis in subjects homozygous or heterozygous for the C282Y mutation. *Gastroenterology 114(5):1003-8,* 1998.	Not a screening population
Cundy T, Bomford A, Butler J, Wheeler M, Williams R. Hypogonadism and sexual dysfunction in hemochromatosis: the effects of cirrhosis and diabetes. *Journal of Clinical Endocrinology & Metabolism 69(1):110-6,* 1989.	Not a screening population
Deugnier YM, Charalambous P, le Quilleuc D, Turlin B, Searle J, Brissot P et al. Preneoplastic significance of hepatic iron-free foci in genetic hemochromatosis: a study of 185 patients. *Hepatology 18(6):1363 -9,* 1993.	Not a screening population
Distante S, Berg JP, Lande K, Haug E, Bell H. HFE gene mutation (C282Y) and phenotypic expression among a hospitalised population in a high prevalence area of haemochromatosis. *Gut.47(4):575-9*, 2000.	Inconsistent application of exclusion criteria
Edwards CQ, Griffen LM, Kushner JP. The morbidity of hemochromatosis among clinically unselected homozygotes: preliminary report. Advances in *Experimental Medicine & Biology.356:303-8,* 1994.	Does not report relevant outcomes

Study Citation	Reason for exclusion
Edwards CQ, Griffen LM, Kushner JP. Comparison of stainable liver iron between symptomatic and asymptomatic hemochromatosis homozygotes and their homozygous relatives. *American Journal of the Medical Sciences.301(1):44-6,* 1991.	Not a screening population
Edwards CQ, Griffen LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP. Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. *New England Journal of Medicine 318 (21):1355-1362*, 1988.	Does not include C282Y genotyping in screening sequence
Edwards CQ, Cartwright GE, Skolnick MH, Amos DB. Homozygosity for hemochromatosis: clinical manifestations. *Annals of Internal Medicine.93(4):519-25*, 1980.	Does not report relevant outcomes
Elliott R, Lin BP, Dent OF, Tait A, Smith CI. Prevalence of hemochromatosis in a random sample of asymptomatic men. *Australian and New Zealand Journal of Medicine 16 (4):491-495,* 1986.	Does not include C282Y genotyping in screening sequence
Elmberg M, Hultcrantz R, Ekbom A, Brandt L, Olsson S, Olsson R et al. Cancer risk in patients with hereditary hemochromatosis and in their first-degree relatives. *Gastroenterology 125(6):1733-41,* 2003.	Not a screened population
Fargion S, Fracanzani AL, Piperno A, Braga M, D'Alba R, Ronchi G et al. Prognostic factors for hepatocellular carcinoma in genetic hemochromatosis. *Hepatology* 1994;(6):1426-1431.	Not a screening population
Fargion S, Mandelli C, Piperno A, Cesana B, Fracanzani AL, Fraquelli M et al. Survival and prognostic factors in 212 Italian patients with genetic hemochromatosis. *Hepatology 15(4):655-9,* 1992.	Not a screening population
Fiel MI, Schiano TD, Bodenheimer HC, Thung SN, King TW, Varma CR et al. Hereditary hemochromatosis in liver transplantation. *Liver Transplantation & Surgery 5(1):50-6,* 1999.	Not a screening population
Fleming DJ, Jacques PF, Tucker KL, Massaro JM, D'Agostino RB, Sr., Wilson PW et al. Iron status of the free-living, elderly Framingham Heart Study cohort: an iron-replete population with a high prevalence of elevated iron stores. *American Journal of Clinical Nutrition 73(3):638-46,* 2001.	Does not report relevant outcomes
Fletcher LM, Dixon JL, Purdie DM, Powell LW, Crawford DH. Excess alcohol greatly increases the prevalence of cirrhosis in hereditary hemochromatosis. *Gastroenterology 122(2):281-9,* 2002.	Not a screening population
Fox CJ, Cullen DJ, Knuiman MW, Cumpston GN, Divitini ML, Rossi E et al. Effects of body iron stores and haemochromatosis genotypes on coronary heart disease outcomes in the Busselton health study. *Journal of Cardiovascular Risk 9(5):287-93,* 2002.	Study disease definition does not meet our definition of asymptomatic primary iron overload or clinical disease.
Fracanzani AL, Conte D, Fraquelli M, Taioli E, Mattioli M, Losco A et al. Increased cancer risk in a cohort of 230 patients with hereditary hemochromatosis in comparison to matched control patients with non-iron-related chronic liver disease. *Hepatology 33(3):647-51,* 2001.	Not a screening population

Appendix C Table 2 (continued). Studies excluded from key question 1.

Study Citation	Reason for exclusion
Fracanzani AL, Fargion S, Romano R, Conte D, Piperno A, D'Alba R et al. Portal hypertension and iron depletion in patients with genetic hemochromatosis. *Hepatology 22(4 Pt 1):1127-31,* 1995.	Not a screening population
Gleeson F, Ryan E, Barrett S, Crowe J. Clinical expression of haemochromatosis in Irish C282Y homozygotes identified through family screening. *European Journal of Gastroenterology & Hepatology 16(9):859-63,* 2004.	Includes data from patients < 18 yrs
Hallberg L, Bjorn-Rasmussen E, Jungner I. Prevalence of hereditary haemochromatosis in two Swedish urban areas. *Journal of Internal Medicine 225 (4):249-255,* 1989.	Does not include C282Y genotyping in screening sequence
Halliday JW, Russo AM, Cowlishaw JL, Powell LW. Serum-ferritin in diagnosis of haemochromatosis. A study of 43 families. *Lancet.2(8039.):621-4*, 1977.	Does not report relevant outcomes
Hamilton EB, Bomford AB, Laws JW, Williams R. The natural history of arthritis in idiopathic haemochromatosis: progression of the clinical and radiological features over ten years. *Quarterly Journal of Medicine 50(199):321-9,* 1981.	Not a screening population
Jackson HA, Carter K, Darke C, Guttridge MG, Ravine D, Hutton RD et al. HFE mutations, iron deficiency and overload in 10,500 blood donors. *British Journal of Haematology 114(2):474-84,* 2001.	Study disease definition does not meet our definition of asymptomatic primary iron overload or clinical disease.
Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. *JAMA 291(6):711-7,* 2004.	Study design
Jonsson JJ, Johannesson GM, Sigfusson N, Magnusson B, Thjodleifsson B, Magnusson S. Prevalence of iron deficiency and iron overload in the adult Icelandic population. *Journal of Clinical Epidemiology 44 (12):1289-1297*, 1991.	Does not include C282Y genotyping in screening sequence
Jorquera F, Dominguez A, Diaz-Golpe V, Espinel J, Munoz F, Herrera A et al. C282Y and H63D mutations of the haemochromatosis gene in patients with iron overload. *Revista Espanola de Enfermedades Digestivas 93(5):293-302,* 2001.	Not a screening population
Karlsson M, Ikkala E, Reunanen A, Takkunen H, Vuori E, Makinen J. Prevalence of hemochromatosis in Finland. *Acta Medica Scandinavica* 224 (4):385-390, 1988.	Does not include C282Y genotyping in screening sequence
Koefoed P, Dalhoff K, Dissing J, Kramer I, Milman N, Pedersen P et al. HFE mutations and hemochromatosis in Danish patients admitted for HFE genotyping. *Scandinavian Journal of Clinical & Laboratory Investigation 62(7):527-35,* 2002.	Not a screening population
Lalouel JM, Le Mignon L, Simon M, Fauchet R, Bourel M, Rao DC et al. Genetic analysis of idiopathic hemochromatosis using both qualitative (disease status) and quantitative (serum iron) information. *American Journal of Human Genetics 37(4):700-18,* 1985.	Does not report relevant outcomes
Leggett BA, Halliday JW, Brown NN, Bryant S, Powell LW. Prevalence of haemochromatosis amongst asymptomatic Australians. *British Journal of Haematology 74 (4):525-530*, 1990.	Does not include C282Y genotyping in screening sequence

Study Citation	Reason for exclusion
Lin E, Adams PC. Biochemical liver profile in hemochromatosis. A survey of 100 patients. *Journal of Clinical Gastroenterology 13(3):316-20,* 1991.	Not a screening population
Lindmark B, ErikssonS. Regional differences in the idiopathic hemochromatosis gene frequency in Sweden. *Acta Medica Scandinavica 218 (3):299-304*, 1985.	Does not include C282Y genotyping in screening sequence
Livesey KJ, Wimhurst VL, Carter K, Worwood M, Cadet E, Rochette J et al. The 16189 variant of mitochondrial DNA occurs more frequently in C282Y homozygotes with haemochromatosis than those without iron loading. *Journal of Medical Genetics 41(1):6-10,* 2004.	Not a screening population
Mainous AG III, Gill JM, Pearson WS. Should we screen for hemochromatosis? An examination of evidence of downstream effects on morbidity and mortality. *Archives of Internal Medicine 162(15):1769-74,* 2002;-26.	Does not report relevant outcomes
Mainous AG III, King DE, Pearson WS, Garr DR. Is an elevated serum transferrin saturation associated with the development of diabetes? *J Fam Pract* 51 (11):933-936, 2002.	Does not include C282Y genotyping in screening sequence
Mainous AG III, Wells B, Carek PJ, Gill JM, Geesey ME. The mortality risk of elevated serum transferrin saturation and consumption of dietary iron. *Annals of Family Medicine.2(2):139-44,*-Apr, 2004.	Does not include C282Y genotyping in screening sequence
Mainous AG III, Gill JM, Carek PJ. Elevated serum transferrin saturation and mortality. *Ann Fam Med* 2 (2):133-138, 2004.	Does not include C282Y genotyping in screening sequence
Mainous AG III, Gill JM, Everet CJ. Transferrin saturation, dietary iron intake, and risk of cancer. *Annals of Family Medicine.3(2):131-7,*-Apr, 2005.	Does not report relevant outcomes
Mathews JL, Williams HJ. Arthritis in hereditary hemochromatosis. *Arthritis & Rheumatism 30(10):1137-41,* 1987.	Study design
McCune CA, Al Jader LN, May A, Hayes SL, Jackson HA, Worwood M. Hereditary haemochromatosis: only 1% of adult HFEC282Y homozygotes in South Wales have a clinical diagnosis of iron overload. *Human Genetics.*111(6):538-43, 2002.	Not a screening population
McCune CA, Ravine D, Worwood M, Jackson HA, Evans HM, Hutton D. Screening for hereditary haemochromatosis within families and beyond. *Lancet 362(9399):1897 -8,* 2003.	Not a screening population Quality
Merryweather-Clarke AT, Worwood M, Parkinson L, Mattock C, Pointon JJ, Shearman JD et al. The effect of HFE mutations on serum ferritin and transferrin saturation in the Jersey population. *British Journal of Haematology 101(2):369-73,* 1998.	Does not report relevant outcomes
Milman N, Pedersen P, Steig T, Byg KE, Graudal N, Fenger K. Clinically overt hereditary hemochromatosis in Denmark 1948-1985: epidemiology, factors of significance for long-term survival, and causes of death in 179 patients. *Annals of Hematology 80(12):737-44,* 2001.	Quality
Milman N. Iron status markers in hereditary haemochromatosis: distinction between individuals being homozygous and heterozygous for the haemochromatosis allele. *European Journal of Haematology 47(4):292-8,* 1991.	Does not report relevant outcomes

Appendix C Table 2 (continued). Studies excluded from key question 1.

Study Citation	Reason for exclusion
Moirand R, Jouanolle AM, Brissot P, Le Gall JY, David V, Deugnier Y. Phenotypic expression of HFE mutations: a French study of 1110 unrelated iron-overloaded patients and relatives. *Gastroenterology 116(2):372-7,* 1999.	Does not report relevant outcomes
Moodie SJ, Ang L, Stenner JM, Finlayson C, Khotari A, Levin GE et al. Testing for haemochromatosis in a liver clinic population: relationship between ethnic origin, HFE gene mutations, liver histology and serum iron markers. *European Journal of Gastroenterology & Hepatology 14(3):223-9,* 2002.	Not a screening population
Morrison ED, Brandhagen DJ, Phatak PD, Barton JC, Krawitt EL, El Serag HB et al. Serum ferritin level predicts advanced hepatic fibrosis among U.S. patients with phenotypic hemochromatosis. *Annals of Internal Medicine 138(8):627-33,* 2003.	Not a screening population
Mura C, Nousbaum JB, Verger P, Moalic MT, Raguenes O, Mercier AY et al. Phenotype-genotype correlation in haemochromatosis subjects. *Human Genetics 101(3):271-6,* 1997.	Not a screening population
Nash S, Marconi S, Sikorska K, Naeem R, Nash G. Role of liver biopsy in the diagnosis of hepatic iron overload in the era of genetic testing. *American Journal of Clinical Pathology 118(1):73-81,* 2002.	Not a screening population
Nelson RL, Persky V, Davis F, Becker E. Risk of disease in siblings of patients with hereditary hemochromatosis. *Digestion.64(2):120-4,* 2001.	Quality
Niederau C, Niederau CM, Lange S, Littauer A, Abdel-Jalil N, Maurer M, Haussinger D, Strohmeyer G. Screening for hemochromatosis and iron deficiency in employees and primary care patients in Western Germany. *Annals of Internal Medicine 128 (5):337-345*, 1998.	Does not include C282Y genotyping in screening sequence
Olsson KS, Eriksson K, Ritter B, Heedman PA. Screening for iron overload using transferrin saturation. *Acta Medica Scandinavica215 (2):105-112*, 1984.	Does not include C282Y genotyping in screening sequence
Olsson KS, Ritter B, Lundin PM. Liver affection in iron overload studied with serum ferritin and serum aminotransferases. *Acta Medica Scandinavica 217(1):79-84,* 1985.	Not a screening population
Olynyk JK, Luxon BA, Britton RS, Bacon BR. Hepatic iron concentration in hereditary hemochromatosis does not saturate or accurately predict phlebotomy requirements. *American Journal of Gastroenterology 93(3):346-50,* 1998.	Does not report relevant outcomes
Panajotopoulos N, Piperno A, Conte D, Mandelli C, Cesana M, Mercuriali F et al. HLA typing in 67 Italian patients with idiopathic hemochromatosis and their relatives. *Tissue Antigens 33(4):431-6,* 1989.	Study design
Phatak PD, Sham RL, Raubertas RF, Dunnigan K, O'Leary MT, Braggins C, Cappuccio JD. Prevalence of hereditary hemochromatosis in 16031 primary care patients. *Annals of Internal Medicine 129 (11):954-961*, 1998.	Does not include C282Y genotyping in screening sequence
Piperno A, Vergani A, Salvioni A, Trombini P, Vigano M, Riva A et al. Effects of venesections and restricted diet in patients with the insulin-resistance hepatic iron overload syndrome. *Liver International 24(5):471-6,* 2004.	Not a screening population

Study Citation	Reason for exclusion
Porto G, Vicente C, Fraga J, da Silva BM, de Sousa M. Importance of establishing appropriate local reference values for the screening of hemochromatosis: a study of three different control populations and 136 hemochromatosis family members. Hemochromatosis Clinical and Research Group. *Journal of Laboratory & Clinical Medicine.119(3):295-305*, 1992.	Includes data from patients < 18 yrs
Porto G, Vicente C, Teixeira MA, Martins O, Cabeda JM, Lacerda R, Goncalves C, Fraga J, Macedo G, Silva BM, Alves H, Justica B,de Sousa M. Relative impact of HLA phenotype and CD4-CD8 ratios on the clinical expression of hemochromatosis. *Hepatology.25(2):397-402,* 1997.	Not a screening population
Poullis A, Moodie SJ, Ang L, Finlayson CJ, Levin GE, Maxwell JD. Routine transferrin saturation measurement in liver clinic patients increases detection of hereditary haemochromatosis. *Annals of Clinical Biochemistry 40(Pt 5):521-7,* 2003.	Not a screening population
Powell LW, Summers KM, Board PG, Axelsen E, Webb S, Halliday JW. Expression of hemochromatosis in homozygous subjects. Implications for early diagnosis and prevention. *Gastroenterology 98(6):1625-32,* 1990.	Includes data from patients < 18 yrs
Poynard T, Mathurin P, Lai CL, Guyader D, Poupon R, Tainturier MH et al. A comparison of fibrosis progression in chronic liver diseases. *Journal of Hepatology 38(3):257-65,* 2003.	Not a screening population
Press RD, Flora K, Gross C, Rabkin JM, Corless CL. Hepatic iron overload: direct HFE (HLA-H) mutation analysis vs quantitative iron assays for the diagnosis of hereditary hemochromatosis. *American Journal of Clinical Pathology 109(5):577-84,* 1998.	Not a screening population
Rhodes DA, Raha-Chowdhury R, Cox TM, Trowsdale J. Homozygosity for the predominant Cys282Tyr mutation and absence of disease expression in hereditary haemochromatosis. *Journal of Medical Genetics 34(9):761-4,* 1997.	Does not report relevant outcomes
Roberts AG, Whatley SD, Morgan RR, Worwood M, Elder GH. Increased frequency of the haemochromatosis Cys282Tyr mutation in sporadic porphyria cutanea tarda. *Lancet 349(9048):321-3,* 1997.	Does not report relevant outcomes
Rossi E, Henderson S, Chin CY, Olynyk J, Beilby JP, Reed WD et al. Genotyping as a diagnostic aid in genetic haemochromatosis. *Journal of Gastroenterology & Hepatology 14(5):427-30,* 1999.	Not a screening population
Rowe JW, Wands JR, Mezey E, Waterbury LA, Wright JR, Tobin J, Andres R. Familial hemochromatosis: characteristics of the precirrhotic stage in a large kindred. *Medicine.56(3):197-211,* 1977.	Does not report relevant outcomes
Ryan E, Byrnes V, Coughlan B, Flanagan AM, Barrett S, O'Keane JC, Crowe J. Underdiagnosis of hereditary haemochromatosis: lack of presentation or penetration? *Gut.51(1):108-12*, 2002.	Includes data from patients < 18 yrs
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 320(7251):1706 -7,* 2000.	Does not report relevant outcomes

Appendix C Table 2 (continued). Studies excluded from key question 1.

Study Citation	Reason for exclusion
Scotet V, Merour MC, Mercier AY, Chanu B, Le Faou T, Raguenes O et al. Hereditary hemochromatosis: effect of excessive alcohol consumption on disease expression in patients homozygous for the C282Y mutation. *American Journal of Epidemiology 158(2):129-34,* 2003.	Does not report relevant outcomes
Sham RL, Ou CY, Cappuccio J, Braggins C, Dunnigan K, Phatak PD. Correlation between genotype and phenotype in hereditary hemochromatosis: analysis of 61 cases. *Blood Cells Molecules & Diseases 23(2):314-20,* 1997.	Not a screening population
Sham RL, Raubertas RF, Braggins C, Cappuccio J, Gallagher M, Phatak PD. Asymptomatic hemochromatosis subjects: genotypic and phenotypic profiles. *Blood 96(12):3707 -11,* 2000.	Not a screening population
Smith BN, Kantrowitz W, Grace ND, Greenberg MS, Patton TJ, Ookubo R, Sorger K, Semeraro JG, Doyle JR, Coope AG, Kamat BR, Maregni LM, Rand WM. Prevalence of hereditary hemochromatosis in a Massachusetts corporation: is Celtic origin a risk factor? *Hepatology 25 (6):1439-1446*, 1997.	Does not include C282Y genotyping in screening sequence
Waalen J, Nordestgaard BG, Beutler E. The penetrance of hereditary hemochromatosis. *Bailliere's Best Practice in Clinical Haematology.*18(2):203-20, 2005.	Review article
Wands JR, Rowe JA, Mezey SE, Waterbury LA, Wright JR, Halliday JW, Isselbacher KJ, Powell LW. Normal serum ferritin concentrations in precirrhotic hemochromatosis. *New England Journal of Medicine.*294(6):302-5, 1976.	Does not report relevant outcomes
Wiggers P, Dalhoj J, Kiaer H, Ring-Larsen H, Petersen PH, Blaabjerg O, Horder M. Screening for haemochromatosis: prevalence among Danish blood donors. *Journal of Internal Medicine 230 (3):265-270*, 1991.	Does not include C282Y genotyping in screening sequence
Willis G, Jennings BA, Goodman E, Fellows IW, Wimperis JZ. A high prevalence of HLA-H 845A mutations in hemochromatosis patients and the normal population in eastern England. *Blood Cells Molecules & Diseases 23(2):288-91,* 1997.	Does not report relevant outcomes
Willis G, Wimperis JZ, Lonsdale R, Fellows IW, Watson MA, Skipper LM et al. Incidence of liver disease in people with HFE mutations. *Gut 46(3):401-4,* 2000.	Does not report relevant outcomes
Willis G, Wimperis JZ, Smith K, Fellows IW, Jennings BA. HFE mutations in the elderly. *Blood Cells Molecules & Diseases 31(2):240-6,* 2003;-Oct.	Study disease definition does not meet our definition of asymptomatic primary iron overload or clinical disease.
Wojcik JP, Speechley MR, Kertesz AE, Chakrabarti S, Adams PC. Natural history of C282Y homozygotes for hemochromatosis. *Canadian Journal of Gastroenterology.*16(5):297-302, 2002.	Not a screening population Includes data from patients < 18 yrs
Yamashita C, Adams PC. Natural history of the C282Y homozygote for the hemochromatosis gene (HFE) with a normal serum ferritin level. *Clin Gastroenterol Hepatol 1 (5):388-391*, 2003.	Not a screening population

Appendix C Table 3. Studies pending assessment for key question 1.

Study Reference

Powell L, Dixon J, Ramm G et al. The penetrance of HFE-associated hemochromatosis as assessed by liver biopsy in subjects identified by health checks, family screening or population screening. *Hepatology* 2004; 40: 574A	Abstract from a meeting. No article published yet.
Falize L, Guillygomarch A. Perrin M, Laine F, Guyader D, Brissot P, Turlin B, Deugnier Y. Reversibility of hepatic fibrosis in treated genetic haemochromatosis a study of 28 cases. *Bioiron Proceedings* (May 2005) P234.	Abstract from a meeting. No article published yet.

Appendix D. Additional study detail for key question 2.

The most relevant data comes from a fair quality retrospective and prospective case series describing cumulative observations on the survival and response to phlebotomy treatment of 251 predominantly male (224/251) patients with hemochromatosis (all with liver biopsies at or after diagnosis to confirm diagnosis) identified in primary care clinics in Germany between 1947 and 1991.[1] About one-fifth of cases were identified through family screening (20/251) or incidental biochemical tests (28/251). Excellent follow-up was achieved, with loss of 2/251 patients (0.8%). Earlier publications described results through 1985.[2,3] From 1979 forward, the diagnostic workup and therapy was protocolized to include liver biopsy with quantified liver iron, repeated phlebotomies until iron depletion as indicated by serum ferritin, and then repeat liver biopsy to confirm iron depletion. It is not clear exactly what proportion of the 251 patients were diagnosed from 1979 forward, although a prior publication from the same group identified 163 patients diagnosed through 1983,[3] suggesting that the results of this study (particularly the longer-term results) predominantly reflect patients diagnosed and treated before the protocolized approach. Timing for liver biopsy is stated to be at the time of diagnosis in these 163 patients, and this paper further states that the diagnosis of hemochromatosis was suspected from clinical features and biochemical tests and established by liver biopsy with histochemical or chemical analysis of hepatic iron concentration in all patients.[3] All patients were treated with phlebotomy to iron depletion, with those after 1979 treated until normal serum ferritin levels were achieved, and iron depletion was confirmed by repeat liver biopsies. Post-treatment biopsies were available for 185 patients (74%), with 34 patients (13.5%) dying before treatment could be completed and the remaining 32 not having completed treatment by the end of the latest study. Cumulative survival for all patients was 93% at 5 years, 77% at 10 years, 62% at 15 years, 55% at 20 years, 46% at 25 years, and 20% at 30 years. Causes of death were not classified as hemochromatosis-related or not. Niederau had excellent follow-up and full ascertainment of cause for all deaths (n=69) during the study with cause of death established by autopsy in 68%, by pre-mortem histology for the 17% of patients with neoplasms, and by clinical reports or accident reports (14%).

At diagnosis, the mean age was 45.7 (SD 10.8) years; 57% (n=142) of patients were cirrhotic (based on liver biopsy interpreted according to generally accepted histological criteria), only 7 of whom were asymptomatic. The proportion that were cirrhotic at diagnosis decreased over time ($p \leq 0.05$), from 80% in 1947-1969 to 49% in 1970-1981 and 41% in 1982-1991. Other measures of disease severity showed similar secular declines. Accordingly, cumulative survival also improved over time. Those diagnosed in 1982-1991 showed better survival over about 10 years of follow-up than the two groups diagnosed earlier (log-rank test, $p \leq 0.05$) and cumulative survival for HC patients diagnosed in 1982-1991 was not significantly reduced from rates expected for an age-and-sex matched population.   Survival differences between subgroups of patients were reported, but are not reliable due to confounding by various factors such as time period of diagnosis, age at diagnosis, sex, excessive alcohol use, and other unmeasured factors such as concomitant hepatitis, dietary factors, etc.

Response to treatment was gauged by comparing pre-post symptoms and clinical features, including level of fibrosis on biopsy, for 185 patients regardless of time of diagnosis.[1] Given the secular trends in symptoms and disease severity, the most meaningful data are those based on objective changes in liver biopsies by degree of fibrosis at baseline. Degree of fibrosis was as follows: Stage 0 (pre-fibrosis, non-fibrosis, only septal fibrosis); Stage 1 (not extensive portal fibrosis without bridging septa); Stage 2 (portal fibrosis with briding septa); Stage 3 (annual fibrosis with vascular disruption and cirrhosis). About half of patients with post-treatment repeat liver biopsies (n=93) had stage 3 fibrosis/cirrhosis at diagnosis; 12 (13%) of these improved to stage 2 fibrosis after treatment, none worsened, and 81 (87%) were unchanged. Among patients with stage 2 fibrosis (n=39), about half (n=20) improved to stage 1, none worsened and about half (n=19) were unchanged. Among those with Stage 1 fibrosis (n=32), one-third showed reversal to pre-fibrosis (stage 0), 1 worsened to Stage 2, and 21 (66%) were unchanged. Among those with no fibrosis at baseline (stage 0), most (20/21) were unchanged, while one patient worsened to Stage 1 fibrosis. Very few patients had liver biopsies showing more severe fibrosis after treatment and liver biopsies after treatment were unchanged for most patients, suggesting that treatment arrested disease progression. However, there is no untreated control group with which to compare these findings. For those with some level of liver fibrosis before treatment, 13-50% showed some improvement, with the lowest proportional improvement seen in those with the most advanced liver disease (stage 3), suggesting that treatment is more effective in earlier disease. However, these findings are based on qualitative histological readings and blinding in outcome assessment was not clearly employed.

A fair quality retrospective case series described long-term survival after phlebotomy treatment to iron depletion in 80/90 (89%) of all hereditary hemochromatosis patients diagnosed between 1958 and 1989 in one regional medical center in Canada.[4] Patients included probands (56%) and family members (44%).[4]

Diagnosis was based on clinical history, physical examination, serum ferritin and % transferrin saturation (excluding other conditions associated with iron overload), and confirmed by liver biopsy. About one-third (n=27) presented with cirrhosis and data are not presented as to whether this proportion changed over the observation period. The time period of diagnosis was not controlled for in any analyses. No patients were reported not to have completed treatment except for those who died before treatment was completed.

Seventeen patients (19%) died during follow-up (mean 8.1 +/- 6.8 follow-up years, range 0-31 years). Fourteen of these patients were probands. Eight patients (8.9%) died prior to completing treatment, three of whom died from presumed hemochromatosis-related causes. Overall, about half of all deaths (n=8) were hemochromatosis-related and the other half (n=9) were unrelated. Cumulative survival in hereditary hemochromatosis patients was 87% at 5 years, 81% at 10 years, and 71% at 20 years and was significantly lower at all time points (except under one year and over 14 years) than the cumulative survival of age-and sex-matched provincial controls taken from 1980-1982 life tables. Use of life-tables not corresponding to the entire time period of diagnosis may have exaggerated the reduced survival seen in patients. Cirrhosis (as opposed to those with fibrosis or normal livers) was significantly related to the risk of death in a multivariate Cox regression analysis controlling for disease and age at diagnosis; this analysis did not control for time period of diagnosis.

A fair-poor quality retrospective case series followed 111 patients over who were diagnosed with idiopathic hemochromatosis through routine practice from about 1935 through 1975 in the U.K.[5] Survival rates and response to phlebotomy treatment to iron depletion were reported for 85 patients (77%). Diagnosis was established by clinical, biochemical and sometimes histological criteria with secondary iron overload excluded. Liver biopsies were repeated after treatment in 75 patients. Six patients (7%) died before treatment could be completed and another 39 (46%) died after treatment completion (7.7 average years between treatment completion and death). Causes of death were not categorized as hemochromatosis related or not. Cumulative survival after diagnosis for treated patients was 66% at 5 years and 32% at 10 years. Post-treatment liver biopsies showed no histological changes in 68 patients (91%), of whom 56 had cirrhosis and 12 had portal fibrosis, improvement from cirrhosis to portal fibrosis in five patients (7%) and progression from portal fibrosis to cirrhosis in 2 patients (3%). Twelve (16%) accumulated enough iron after treatment to require a repeat course of venesection. The study reported comparisons to untreated historical controls (n=26) but these were excluded due to clear or possible non-comparability between the two groups on numerous features and potential confounding by secular trends in treatment improvements for severe disease such as diabetes mellitus or hepatic failure.

References

1. Niederau C, Fischer R, Purschel A, Stremmel W, Haussinger D, Strohmeyer G. Long-term survival in patients with hereditary hemochromatosis. *Gastroenterology 110(4):1107-19,* 1996.

2. 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. *New England Journal of Medicine 313(20):1256-62,* 1985.

3. Strohmeyer G, Niederau C, Stremmel W. Survival and causes of death in hemochromatosis. Observations in 163 patients. *Annals of the New York Academy of Sciences 526 :245-57,* 1988.

4. Adams PC, Speechley M, Kertesz AE. Long-term survival analysis in hereditary hemochromatosis. *Gastroenterology 101(2):368-72,* 1991.

5. Bomford A, Williams R. Long term results of venesection therapy in idiopathic haemochromatosis. *Quarterly Journal of Medicine 45(180):611-23,* 1976.

Appendix D Table 1. Therapeutic phlebotomy studies.

Study Ref	Setting Study Design	Population	Inclusion Criteria	Control Group	Follow-up
Adams, 1991[25]	Specialty clinic Canada Retrospective case series	n=85 Proband: 48 Discovered family member: 37 Male: 53 Arthritis: 40 Diabetes: 18	Diagnosed between 1958-1989. Diagnosis was based on clinical history, physical examination, serum ferritin and transferring saturation. It was confirmed through liver biopsy. Patients with iron-loading anemias, transfusional iron overload and dietary iron overload were excluded.	Survival was compared against provincial life-table data matched for age and sex.	Mean: 8.1 ± 6.8 yrs Analysis was censored at 20 yrs because only 5 patients were followed for more than 20 yrs.
Bomford, 1976[69]	Specialty Clinic U.K. Case-series	Patients diagnosed through routine clinical practice who received treatment n = 111 Treated: n = 85 Untreated controls: n = 26	Excluded those with secondary iron overload. Diagnosis made "by clinical, biochemical and where possible histological criteria."	26 untreated historical controls that were not comparable to treated subjects.	1937-approx. 1975

Study Ref	Treatment	Measure	Results	Adverse Events	Quality

Adams, 1991[25]	500 mL blood/week until serum ferritin < 30µg/L or patient became anemic. Mean number of treatments: 43 + 51
	
	Treatment resumed if serum ferritin levels became elevated.
		Deaths:
		Cumulative survival:
		  at 5 yrs
		  at 10 yrs
		  at 20 yrs
		Expected survival:
		
		Adjusted relative risk of death:
		  Cirrhosis
		  Arthritis
			17
			
			87%
			81%
			71%
			Significantly decreased survival at all times except 1 yr and >14 yrs.
			
			There was no significant difference between noncirrhotic patients and a hypothetical cohort of age- and sex-matched patients.
			
			5.54
			0.24
				Not reported	Fair

Bomford, 1976[69]	600 mL were removed weekly until hemoglobin ≤ 10 g/dl and serum iron to below 10 µmol/l. Biopsy usually repeated after completion of treatment. Treatment resumed if chelatable body iron levels increased to more than 1000 µg/kg
	79/85 completed full course.
		Diabetes:
		  Improved
		  Worsened
		  New cases
		
		Liver Histology:
		  Improved
		  No definite change
		  Worsened
			
			16/56
			7/56
			3
			
			n=75
			5/75
			68/75
			2/75
				Not reported	Fair

Appendix D Table 1 (continued). Therapeutic phlebotomy studies.

Study Ref	Setting Study Design	Population	Inclusion Criteria	Control Group	Follow-up

Niederau, 1996[26]	Primary care clinics diagnosed
	
	Germany
	
	Retrospective case-series
		n=251
		Age: 45.7 + 10.8 yr
		Male: 224
		Noncirrhotic: 109
		  Asymptomatic: 41
		  Family screening: 15
		Cirrhotic: 142
		  Asymptomatic: 7
		Diabetic: 120
		
		2 were lost to follow-up
			Diagnosed between 1947 and 1991.
			
			Patients were diagnosed on clinical features and biochemical tests: liver function, serum iron, transferrin saturation and serum ferritin. Confirmed by liver biopsy.
				Expected deaths were calculated for a German normal population that was age- and sex-matched for time period of observation.	Mean: 14.1 + 6.8 yr

Appendix D Table 1 (continued). Therapeutic phlebotomy studies.

Study Ref	Treatment	Measure	Results				Adverse Events	Quality

Niederau, 1996[26]	From 1979 on, patients were treated once to twice weekly by phlebotomy of 500 Ml until serum ferritin levels were normal.	Cumulative survival:
		  at 5 yrs
		  at 10 yrs
		  at 20 yrs
		  at 30 yrs
			93%
			77%
			55%
			20%
			Significantly reduced compared with expected survival in matched population.
							Not reported	Fair

185 patients with documented iron depletion received 84.8 + 4.4 treatments to achieve depletion.

All patients received 4-12 phlebotomies per yr after depletion.

Liver iron concentration at diagnosis

Fibr stage	# of pts	Liver iron
0	7	11.6 + 1.8
1	10	13.9 + 1.1
2	9	16.9 + 1.4
3	15	22.4 + 2.0
All	41	16.1 + 1.6

Changes in fibrosis stage after iron depletion

Stage	I	W	U
0	0	1	20
1	10	1	21
2	20	0	19
3	12	0	81

I-improved, U-unchanged, W-worsened

	AD%	I%	U%	W%
Weakness/lethargy	80	55	40	6
Abdominal pain	56	68	29	1
Arthralgia	45	30	50	20
Elevated AST or ALT	81	73	25	2
Pigmentation	68	68	32	0
Loss of potency (163 men)	40	19	69	12
Electrocardiographic changes	35	34	61	5
Diabetes mellitus	44	41	53	6
Impaired glucose tolerance	15	37	56	7

AD-at diagnosis, I-improved, U-unchanged, W-worsened

Appendix D Table 1 (continued). Therapeutic phlebotomy studies.

Study Ref	Setting Study Design	Population	Inclusion Criteria	Control Group	Follow-up

McDonnell, 1999[65]	Population-based mailings to all known patients with hemochromatosis and to organizations with access to hemochromatosis patients in U.S., Canada, Australia, and northern Europe.
	
	From at least 17 countries including U.S. (84%), Australia (6%), U.K. (6%), Canada (4%)
	
	Retrospective cross-sectional
		2,851 patients (80% of all surveys mailed)
		White: 99%
		Male: 62%
		Diagnosis 1990 or later: 70%
		Diagnosis < 1980: 6%
			Led to diagnosis: 35% symptoms related to HH, 45% routine or ancillary lab test, 20% from diagnosis of family member.
			
			56% were diagnosed by primary care physician.
			
			67% had been initially diagnosed with alternate condition to explain symptoms.
			
			Mean age onset of symptoms:
			  41 $\pm$ 14 yrs
			Mean age sought treatment:
			  43 $\pm$ 14 yrs
			Mean age diagnosis:
			  50 $\pm$ 13 yrs
					Not applicable

Study Ref	Treatment	Measure	Results	Adverse Events	Quality
McDonnell, 1999[65]	Location received phlebotomy: doctor's office/hospital (73%), blood bank (25%), their home (0.1%)	Some or all of symptoms improved with therapy: Average time for improvement: New symptoms developed despite treatment:	86% 39 ± 67 weeks 33%	65% of patients with symptoms said the benefit of treatment outweighed the difficulties. 20% found the process routine and expressed indifference. 12% expressed a negative attitude toward phlebotomy that they attributed to poor venous access, time involved, dissatisfaction the removed blood was discarded.	Fair
				RS(%)	IT(%)	WT(%)		
		Extreme fatigue: Joint pain: Impotence or loss of libido: Skin bronzing: Heart fluttering: Depression: Abdominal pain:	1,296(45.5) 705(54.4) 223(17.2) 1,241(43.5) 115(9.2) 422(34.0) 735(25.8) 93(12.7) 204(27.8) 733(25.7) 431(58.8) 30(4.1) 679(23.8) 42(6.2) 69(10.1) 592(20.8) 242(40.8) 61(10.3) 578(20.3) 129(22.3) 69(11.9) RS-reported symptom, IT-improved with therapy, WT-worse despite therapy Compared against NHANES II, III similar proportion of patients reported arthritis, liver or gallbladder disease, extreme fatigue as general population.		

Appendix D Table 2. Studies excluded from key question 2.

Study Citation	Reason for exclusion
Adams PC, Kertesz AE, Valberg LS. Rate of iron reaccumulation following iron depletion in hereditary hemochromatosis. Implications for venesection therapy. *Journal of Clinical Gastroenterology 16(3):207-10,* 1993.	Does not present relevant outcomes
Adams PC. Factors affecting the rate of iron mobilization during venesection therapy for genetic hemochromatosis. *American Journal of Hematology 58(1):16-9,* 1998.	Does not present relevant outcomes
Askari AD, Muir WA, Rosner IA, Moskowitz RW, McLaren GD, Braun WE. Arthritis of hemochromatosis. Clinical spectrum, relation to histocompatibility antigens, and effectiveness of early phlebotomy. *American Journal of Medicine 75(6):957 -65,* 1983.	Quality
Barton JC, Bottomley SS. Iron deficiency due to excessive therapeutic phlebotomy in hemochromatosis. *American Journal of Hematology 65(3):223-6,* 2000.	< 20 patients
Batey RG, Hussein S, Sherlock S, Hoffbrand AV. The role of serum ferritin in the management of idiopathic haemochromatosis. *Scandinavian Journal of Gastroenterology 13(8):953 -7,* 1978.	Does not present relevant outcomes
Bodemann HH, Tanzi-Fetta RF, Schroter-Urban H, Volk BA, Keul J, Lohr GW. Ferritin in erythrocytes and plasma of patients with iron overload. *Blut 51(1):25-31,* 1985.	Quality
Candell-Riera J, Lu L, Seres L, Gonzalez JB, Batlle J, Permanyer-Miralda G et al. Cardiac hemochromatosis: beneficial effects of iron removal therapy. An echocardiographic study. *American Journal of Cardiology 52(7):824-9,* 1983.	Quality
Cesana M, Mandelli C, Tiribelli C, Bianchi PA, Conte D. Concomitant primary hemochromatosis and beta-thalassemia trait: iron depletion by erythrocytapheresis and desferrioxamine. *American Journal of Gastroenterology 84(2):150-2,* 1989.	< 20 patients
Chow LH, Frei JV, Hodsman AB, Valberg LS. Low serum 25-hydroxyvitamin D in hereditary hemochromatosis: relation to iron status. *Gastroenterology 88(4):865-9,* 1985.	Quality
Cleton MI, de Bruijn WC, van Blokland WT, Marx JJ, Roelofs JM, Rademakers LH. Iron content and acid phosphatase activity in hepatic parenchymal lysosomes of patients with hemochromatosis before and after phlebotomy treatment. *Ultrastructural Pathology 12(2):161-74,* 1988;-Apr.	< 20 patients
Cleton MI, Roelofs JM, Blok-Van Hoek CJ, de Bruijn WC. Integrated image and X-ray microanalysis of hepatic lysosomes in a patient with idiopathic hemosiderosis before and after treatment by phlebotomy. *Scanning Electron Microscopy (Pt 3):999-1006,* 1986.	< 20 patients
Conte D, Mandelli C, Cesana M, Ferrini R, Marconi M, Bianchi A. Effectiveness of erythrocytapheresis in idiopathic hemochromatosis. Report of 14 cases. *International Journal of Artificial Organs 12(1):59-62,* 1989.	Does not report relevant outcomes
Conte D, Piperno A, Mandelli C, Fargion S, Cesana M, Brunelli L et al. Clinical, biochemical and histological features of primary haemochromatosis: a report of 67 cases. *Liver 6(5):310-5,* 1986.	Quality

Appendix D Table 2 (continued). Studies excluded from key question 2.

Study Citation	Reason for exclusion
Cundy T, Butler J, Bomford A, Williams R. Reversibility of hypogonadotrophic hypogonadism associated with genetic haemochromatosis. *Clinical Endocrinology 38(6):617-20,* 1993.	< 20 patients
Dabestani A, Child JS, Henze E, Perloff JK, Schon H, Figueroa WG et al. Primary hemochromatosis: anatomic and physiologic characteristics of the cardiac ventricles and their response to phlebotomy. *American Journal of Cardiology 54(1):153-9,* 1984.	< 20 patients
Dymock IW, Cassar J, Pyke DA, Oakley WG, Williams R. Observations on the pathogenesis, complications and treatment of diabetes in 115 cases of haemochromatosis. *American Journal of Medicine 52(2):203-10,* 1972.	Quality
Easley RM, Jr., Schreiner BF, Jr., Yu PN. Reversible cardiomyopathy associated with hemochromatosis. *New England Journal of Medicine 287(17):866 -7,* 1972.	< 20 patients
Failla M, Giannattasio C, Piperno A, Vergani A, Grappiolo A, Gentile G et al. Radial artery wall alterations in genetic hemochromatosis before and after iron depletion therapy. *Hepatology 32(3):569-73,* 2000.	< 20 patients
Fargion S, Mandelli C, Piperno A, Cesana B, Fracanzani AL, Fraquelli M, Bianchi PA, Fiorelli G, Conte D. Survival and prognostic factors in 212 Italian patients with genetic hemochromatosis. *Hepatology.15(4):655-9*, 1992.	Quality
Feely J, Counihan TB. Haemochromatosis presenting as angina and responding to venesection. *British Medical Journal 2(6088):681-2,* 1977.	< 20 patients
Fellows IW, Stewart M, Jeffcoate WJ, Smith PG, Toghill PJ. Hepatocellular carcinoma in primary haemochromatosis in the absence of cirrhosis. *Gut 29(11):1603 -6,* 1988.	< 20 patients
Fracanzani AL, Fargion S, Romano R, Conte D, Piperno A, D'Alba R et al. Portal hypertension and iron depletion in patients with genetic hemochromatosis. *Hepatology 22(4 Pt 1):1127-31,* 1995.	Quality
Gama R, Smith MJ, Wright J, Marks V. Hypopituitarism in primary haemochromatosis; recovery after iron depletion. *Postgraduate Medical Journal 71(835):297-8,* 1995.	< 20 patients
Goh J, Callagy G, McEntee G, O'Keane JC, Bomford A, Crowe J. Hepatocellular carcinoma arising in the absence of cirrhosis in genetic haemochromatosis: three case reports and review of literature. *European Journal of Gastroenterology & Hepatology 11(8):915-9,* 1999.	< 20 patients
Grima KM. Therapeutic apheresis in hematological and oncological diseases. *Journal of Clinical Apheresis 15(1-2):28-52,* 2000.	Review article
Guillygomarc'h A, Mendler MH, Moirand R, Laine F, Quentin V, David V, Brissot P, Deugnier Y. Venesection therapy of insulin resistance-associated hepatic iron overload. Journal of Hepatology.35(3):344-9, 2001.	Wrong population
Hash RB. Hereditary hemochromatosis. *Journal of the American Board of Family Practice 14(4):266 -73,* 2001;-Aug.	Review article

Appendix D Table 2 (continued). Studies excluded from key question 2.

Study Citation	Reason for exclusion
Hines C, Jr., Davis WD, Jr., Ferrante WA. Hepatoma developing in hemochromatosis in spite of adequate treatment by multiple phlebotomies. *American Journal of Digestive Diseases 16(4):349-55,* 1971.	Case report
Hramiak IM, Finegood DT, Adams PC. Factors affecting glucose tolerance in hereditary hemochromatosis. *Clinical & Investigative Medicine - Medecine Clinique et Experimentale* 1920;(2):110-118.	Quality
Hultcrantz R, Angelin B, Bjorn-Rasmussen E, Ewerth S, Einarsson K. Biliary excretion of iron and ferritin in idiopathic hemochromatosis. *Gastroenterology 96(6):1539 -45,* 1989.	Quality
Jakeman A, Thompson T, McHattie J, Lehotay DC. Sensitive method for nontransferrin-bound iron quantification by graphite furnace atomic absorption spectrometry. *Clinical Biochemistry 34(1):43-7,* 2001.	< 20 patients
Kaltwasser JP, Werner E, Schalk K, Hansen C, Gottschalk R, Seidl C. Clinical trial on the effect of regular tea drinking on iron accumulation in genetic haemochromatosis. *Gut 43(5):699-704,* 1998.	Quality
Kelly TM, Edwards CQ, Meikle AW, Kushner JP. Hypogonadism in hemochromatosis: reversal with iron depletion. *Annals of Internal Medicine 101(5):629-32,* 1984.	Does not present relevant outcomes
Kohan A, Niborski R, Daruich J, Rey J, Bastos F, Amerise G, Herrera R, Garcia M, Olivera W, Santarelli MT, Avalos JS, Findor J. Erythrocytapheresis with recombinant human erythropoietin in hereditary hemochromatosis therapy: a new alternative. Vox.Sanguinis.79(1):40-5, 2000.	< 20 patients
Leitman SF, Browning JN, Yau YY, Mason G, Klein HG, Conry-Cantilena C et al. Hemochromatosis subjects as allogeneic blood donors: a prospective study. *Transfusion 43(11):1538 -44,* 2003.	Does not report relevant outcomes
Limdi JK, Crampton JR. Hereditary haemochromatosis. *Qjm 97(6):315-24,* 2004.	Review article
Lombard M, Bomford A, Hynes M, Naoumov NV, Roberts S, Crowe J et al. Regulation of the hepatic transferrin receptor in hereditary hemochromatosis. *Hepatology 9(1):1-5,* 1989.	Does not present relevant outcomes
Lufkin EG, Baldus WP, Bergstralh EJ, Kao PC. Influence of phlebotomy treatment on abnormal hypothalamic-pituitary function in genetic hemochromatosis. *Mayo Clinic Proceedings 62(6):473-9,* 1987.	Quality
Mainous AG, III, Wells B, Carek PJ, Gill JM, Geesey ME. The mortality risk of elevated serum transferrin saturation and consumption of dietary iron. *Annals of Family Medicine 2(2):139-44,* 2004;-Apr.	No phlebotomy treatment
Mandelli C, Cesarini L, Piperno A, Fargion S, Fracanzani AL, Barisani D et al. Saturability of hepatic iron deposits in genetic hemochromatosis. *Hepatology 16(4):956 -9,* 1992.	Does not present relevant outcomes
McDonnell SM, Witte DL, Cogswell ME, McIntyre R. Strategies to increase detection of hemochromatosis. *Annals of Internal Medicine 129(11):987 -92,* 1998.	Review article

Appendix D Table 2 (continued). Studies excluded from key question 2.

Study Citation	Reason for exclusion
Milman N, Pedersen P, Steig T, Byg KE, Graudal N, Fenger K. Clinically overt hereditary hemochromatosis in Denmark 1948-1985: epidemiology, factors of significance for long-term survival, and causes of death in 179 patients. *Annals of Hematology 80(12):737-44,* 2001.	Quality
Milman N. Hereditary haemochromatosis in Denmark 1950-1985. Clinical, biochemical and histological features in 179 patients and 13 preclinical cases. *Danish Medical Bulletin 38(4):385-93,* 1991.	Does not report relevant outcomes
Moirand R, Adams PC, Bicheler V, Brissot P, Deugnier Y. Clinical features of genetic hemochromatosis in women compared with men. *Annals of Internal Medicine 127(2):105-10,* 1997.	Does not report relevant outcomes
Morcos M, Dubois S, Bralet MP, Belghiti J, Degott C, Terris B. Primary liver carcinoma in genetic hemochromatosis reveals a broad histologic spectrum. *American Journal of Clinical Pathology 116(5):738 -43,* 2001.	Does not report relevant outcomes
Muncunill J, Vaquer P, Galmes A, Obrador A, Parera M, Bargay J, Besalduch J. In hereditary hemochromatosis, red cell apheresis removes excess iron twice as fast as manual whole blood phlebotomy. Journal of Clinical Apheresis.17(2):88-92, 2002.	< 20 patients
Muting D, Kalk JF, Fischer R, Wiewel D. Spontaneous regression of oesophageal varices after long-term conservative treatment. Retrospective study in 20 patients with alcoholic liver cirrhosis, posthepatitic cirrhosis and haemochromatosis with cirrhosis. *Journal of Hepatology 10(2):158-62,* 1990.	Not phlebotomy treatment
Niederau C, Stremmel W, Strohmeyer GW. Clinical spectrum and management of haemochromatosis. *Baillieres Clinical Haematology 7(4):881-901,* 1994.	Review article
Niederau C, Strohmeyer G, Stremmel W. Epidemiology, clinical spectrum and prognosis of hemochromatosis. *Advances in Experimental Medicine & Biology 356:293-302,* 1994.	Review article
Olsson KS, Ritter B, Lundin PM. Liver affection in iron overload studied with serum ferritin and serum aminotransferases. *Acta Medica Scandinavica 217(1):79-84,* 1985.	Quality
Piperno A, Vergani A, Salvioni A, Trombini P, Vigano M, Riva A et al. Effects of venesections and restricted diet in patients with the insulin-resistance hepatic iron overload syndrome. *Liver International 24(5):471-6,* 2004.	Does not report relevant outcomes
Propper R, Nathan D. Clinical removal of iron. *Annual Review of Medicine 33:509-19,* 1982.	Clinical review article
Prunescu CC, Prunescu P, Vilcu AL. Ultrastructure of the liver in idiopathic haemosiderosis and results of a treatment by repeated bleedings. *Morphologie et Embryologie 33(2):133-6,* 1987;-Jun.	Case report
Riquelme A, Soza A, Nazal L, Martinez G, Kolbach M, Patillo A et al. Histological resolution of steatohepatitis after iron depletion. *Digestive Diseases & Sciences 49(6):1012-5,* 2004.	Case report
Sargent T, Saito H, Winchell HS. Iron absorption in hemochromatosis before and after phlebotomy therapy. *Journal of Nuclear Medicine 12(10):660 -7,* 1971.	Does not report relevant outcomes

Study Citation	Reason for exclusion
Seamark CJ, Hutchinson M. Controversy in primary care: Should asymptomatic haemochromatosis be treated? *BMJ 320(7245):1314 -7,* 2000.	Case report
Sigal SH, Fleischner GM, Weiner FR. Hypogonadal-induced anemia in genetic hemochromatosis: implications for phlebotomy therapy. *American Journal of Gastroenterology 90(1):152-3,* 1995.	Case report
Spellberg MA. Treatment of hemochromatosis. *American Journal of Gastroenterology 51(6):516 -22,* 1969.	Review article
Tiniakos G, Williams R. Cirrhotic process, liver cell carcinoma and extrahepatic malignant tumors in idiopathic haemochromatosis. Study of 71 patients treated with venesection therapy. *Applied Pathology 6(2):128-38,* 1988.	Quality
Weintraub LR, Conrad ME, Crosby WH. The treatment of hemochromatosis by phlebotomy. *Medical Clinics of North America 50(6):1579-90,* 1966.	< 20 patients
Wojcik JP, Speechley MR, Kertesz AE, Chakrabarti S, Adams PC. Natural history of C282Y homozygotes for hemochromatosis. *Canadian Journal of Gastroenterology 16(5):297-302,* 2002.	Does not report relevant outcomes

Appendix E Table 1. Studies of high-risk groups for C282Y homozygosity or HH.

Study Ref	Setting Time frame Country	Study Design	Sample	Risk Group Definition	Inclusion Exclusion	Population
Family Studies						
Barton, 1999[67]	Southern Iron Disorder Center and Brookwood Medical Center No dates reported USA	Cross Sectional: To compare phenotyping and *HFE* genotyping for diagnosis of HH in 150 family members of 61 probands	HH probands diagnosed during routine medical care delivery from June 1996-June 1998 (Genetic testing not used to diagnose probands before family members identified-only 73.8% were C282Y/C282Y) 150 family members of 61 probands (did not report what % of total)	Relatives of people with iron overload (probands: 16% had cirrhosis and 5% had diabetes attributable to iron overload)	Inclusion: willingness of probands and a family member to participate Exclusions: NR	72 (48%) males 78 (52%) females Mean age 46 $\pm$ 15 years (all were adults except one 11 year old) 94 were first degree relatives 56 2nd degree non-blood relatives
McCune, 2003[72]	Three health authorities in South Wales No dates reported UK	Cross-sectional: How information about genetic risk is transmitted through families, and to assess uptake of genetic screening within a large population of young health adults.	1st degree relatives of two groups of index cases 180 eligible from screening probands and 143 from clinical probands Probands: 72 individual homozygous for the C282Y mutation from 10,556 blood donors and Patients who presented clinically within three health authorities in South Wales. They were all C282Y homozygous	1st degree relatives of homozygous for *HFE* C282Y	Included: parents and siblings, including those previously tested for HH	163/180 (91%) 1st degree relatives of screening probands 121/143 (85%) 1st degree relatives of clinically diagnosed probands

Study Ref	Initial Screening Sequence	Definition Clinical HC	Diagnostic Criteria	Results	Quality
Family Studies					
Barton, 1999[67]	Simultaneous genetic testing for *HFE* alleles C282Y and H63D; and Phenotype testing using TS; and SF	Phenotype definition: Elevated fasting TS on at least two occasions in the absence of other known causes > 60% M; > 50% F Iron Overload: Elevated SF concentration (> 300 ng/ml in men and > 200 ng/ml in women), increased hepatic iron content determined using hepatic biopsy specimens, or iron > 4g mobilized by TP) Genetic criteria not used.	Hemochromatosis phenotype: presence of elevated transferrin saturation or iron overload or both.	1 and 2 degree relatives C282Y/C282Y = 25/112 (calculated) <u>C282Y/C282Y</u> 14/42 siblings (33%) 3/16 parents (19% 5/36 offspring (14%) 3/18 other blood relatives (16%) 22/61 probands: blood relative with HH (36%) All of these were C282Y/C282Y HH Phenotype: 1st degree Relatives 30/94 (31.9% non-1st degree Relatives 4/56 (7.1%)	Good/ Fair
		Hepatic Cirrhosis	NR	<u>C282/C282Y</u> <u>C282/H63D</u> <u>Other</u> <u>Total</u> 2+0 +0 +0 2/112 (1.7%)	
		DM	NR	4+0 +0 +0 4/112 (3.6%)	
McCune, 2003[72]	Physician interview Blood Tests: TS, SF, *HFE* Genotyping (mutation testing no specified) Abnormal TS >50%, SF >300 µg/L (>200µg/L for premenopausal women)	NR	NA	Relatives of probands from screening C282Y/C282Y = 25/163 (15% ©) (15 women and 10 men) >TS and SF 10/25 (40%) (3 F and 10 M) Relatives of probands from clinically diagnosed C282Y/C282Y = 59/121 (49%(calc)) Previously untested 34/57 (60%(calc)) (25 had already been DNA tested and 34 new cases were identified) >TS and SF 20/34 (59%) (8 F and 12 M)	Fair

Appendix E Table 1 (continued). Studies of high-risk groups for C282Y homozygosity or HH.

Study Ref	Setting Time frame Country	Study Design	Sample	Risk Group Definition	Inclusion Exclusion	Population
Other Targeted Screening						
Cadet, 2003[75]	Multiple settings: Primary care patients were recruited from three Oxfordshire practices and secondary care patients were recruited from those patients attending specialist clinics in Amiens University Hospital No dates reported France	Cohort: To determine the optimal means of identifying patients with undiagnosed HH using *HFE* genotype and/or phenotype	**Primary care** patients 4022 consultations, during which 169 patients were identified with an index symptom (diabetes, arthropathy, unexplained fatigue, abdominal pain, liver disease, abnormal LFT, impotency, premature amenorrhea, or cardiac arrhythmia) of who 88 were aged between 25 and 70 and offered a genetic test for HC; 60 patients were tested **Secondary care:** Several groups of patients attending specialty clinics at a hospital **Rheumatology Clinics:** 221 Rheumatoid factor-negative patients with osteoporosis or arthropathy **Endocrinology Clinics:** 121 diabetic patients from one endocrine dept. (they included unstable diabetes.) **Internal Medicine Clinics**: 227 patients with chronic fatigue and arthralgia **Controls**; recruited from 2337 subjects over 18 from a health Appraisal Center.	Patients with presenting conditions possibly related to hemochromatosis	**Case:** Exclusions: families or patient previously diagnosed with HH **Control:** Inclusion: >18 y Living in Picardy Attended a free health check-up clinic	**Case Group** **Osteoporosis** N=159 Sex NR Age 64+12 years **Arthropathy** N=62 Sex NR Age 61.3+13.9 years **Diabetes** N= 121 F: 42; M: 79 Age 54.8+8.3 years **Fatigue and Arthralgia** N= 227 F: 144; M: 83 Age 58.3+15.6 years **Control Group**: N=991 (random sample of 2337) F: 483, M: 508 Age 42.5+14.9 Osteoporosis was based on radiographical analysis Arthropathy was based on clinical diagnosis Diabetes= three groups, Type 1, type 2, and could not be classified. Fatigue/arthralgia; referral to internal medicine for chronic problem

Study Ref	Initial Screening Sequence	Definition Clinical HC	Diagnostic Criteria	Results							Quality
Other Targeted Screening											

Cadet, 2003[75] — HFE C283Y and H63D mutations, Serum iron, serum ferritin — Definition Clinical HC: NA — Diagnostic Criteria: NA — Quality: Fair

Results:

Genotype		HV N=991	PC N=60	OS N=159	AR N=62	DM N=121	F/A N=227
HH/CC	%	60.9	68.3	56.0	59.7	42.1**	44.9**
HD/CC	%	26.4	23.3	29.0	25.8	24.0	21.6
HH/CY	%	6.8	5.0	10.0	8.0	14.9*	10.6
DD/CC	%	2.7	3.3	2.5	6.5	5.0	9.3**
HD/CY	%	2.9	0	1.9	0	8.3**	7.9*
HH/YY	%	0.2	0	0.6	0	5.8**	5.7**

Genotypes are expressed as percentage. The x^2-test was used to determine the significance in each genotype versus health volunteers (*P<0.01, **P=<0.001)
HH/CC-wild type; HD/CC-H63D heterozygous; HH/CY-C282Y heterozygous; DD/CC-H63D homozygous; HD/CY-compound heterozygous; HH/YY-C282Y homozygous; HV-healthy volunteer; PC-primary care; OS-osteoporosis; AR-arthropathy; DM-diabetes mellitus; F/A-fatigue and arthralgia.

Phenotype	HV N=991	PC N=60	OS + AR N=221	DM N=121	F/A N=227
TS>40%	29.6%	NR	4.1%	87.6%	30.8%
% of >40 are YY	0.07%	NR	11.1%	6.6%	18.6%
# of >40 are YY	(2/293)	NR	(1/9)	(7/106)	(13/70)
SF>300µg/L	5.8%	NR	4.1%	46.3%	33.0%
% of >300 are YY	0%	NR	11.1%	10.7%	17.3%
# of >300 are YY	(0/57)	NR	(1/9)	(6/56)	(13/75)

Appendix E Table 1 (continued). Studies of high-risk groups for C282Y homozygosity or HH.

Study Ref	Setting Time frame Country	Study Design	Sample	Risk Group Definition	Inclusion Exclusion	Population
Other Targeted Screening-continued						
Deugnier, 2002[56]	Men and Women attending Health Appraisal Centres Sept-98 to Dec-2000. France	Cross Sectional:	M aged 25-40, W aged 35-50	Family history of iron excess	Incl: Attending Health Appraisal Centres, meeting age criteria.Those who declined genotyping (4%) had no personal history suggestive of iron excess.	N = 9396 (96% of total population) M: 3367 F: 6029
				Chronic fatigue		
				Increased ALT		

Study Ref	Initial Screening Sequence	Definition Clinical HC	Diagnostic Criteria	Results	Quality
Other Targeted Screening-continued					
Deugnier, 2002[56]	Questionnaire; age, gender, body mass index, awareness of a family relative regularly phlebotomized for iron excess, personal history of blood donation, chronic fatigue, chronic distal arthralgias, diabetes etc)*HFE* C282Y mutation testing, and if C282Y homozygoteFasting Serum iron status (Iron, TS and Ferritin) and genetic counseling	*HFE* C282Y mutation testing	NA	% C282Y homozygotes by Family history of iron excess Males: Family history 3/83 (3.6%) (c) No family history 7/3904 (0.2%) (c) Females: Family history 12/16 (75%) (c) No family history 21/175 (12%) (c) C282Y homozygotes <u>All subjects</u> <u>Males</u> <u>Females</u> 54/9396 (0.006%) 10/3367 (0.003%) 44/6029 (0.007%) % C282Y homozygotes by Chronic fatigue Males: Chronic fatigue 7/828 (0.85%) (calc) No chronic fatigue 3/2180 (0.14%) (calc) Females: Chronic fatigue 12/2253 (0.53%) (calc) No chronic fatigue 28/3361 (0.83%) (calc) % C282Y homozygotes by Increased ALT Males: Increased ALT 1/176 (0.57%) (calc) Not increased 9/3181 (0.28%) (calc) Females: Increased ALT 3/322 (0.62%) (calc) Not increased 42/5694 (0.74%) (calc)	Fair

Study Ref	Setting Time frame Country	Study Design	Sample	Risk Group Definition	Inclusion Exclusion	Population
CHD, Coronary Artery Disease etc						
Waalen, 2002[79]	Health appraisal Center in San Diego Calif May 1999-August 2001 USA	Cross Sectional: examine the relationship between two *HFE* mutations (C282Y and H63D), and the prevalence of CHD in a large white adult population	All white, non-Hispanic, adults patients aged $\geq$25 who attended a Health Appraisal Center visits between May 1999 and August 2001 N= 35,792	History of CHD Defined as "yes" to questions " Have you had a heart attach for which you were hospitalized for at least 3 days?" or "Do you have angina pectoris?", or an ICD9 code 410 or 412 in the medical record.	Inclusion: white, non-Hispanic aged 25-98 attending Health Appraisal Center of an HMO 46% gave consent for *HFE* mutation testing	M= 15,362 F= 15,554 all participants were white, non-hispanic
Rosenqvist 1989[76]	Huddinge University Hospital, Huddinge, Sweden No time frame reported Sweden	Cross Sectional: To assess the prevalence of iron overload among men with clinically significant bradyarrhythmias necessitating treatment with permanent cardiac pacing. No *HFE* testing-HLA done only in biopsied men.	All pacemaker-treated men at the hospital, with a catchment population of about 800,000 Blood specimens obtained from >95% of male pacemaker patients	Male patients with indications for a permanent pacemaker for second to third degree block or sinus node disease	NR	149 with heart block 83 with sinus node disease Mean age at implantation of pacemaker was 73 $\pm$ 10 years

Study Ref	Initial Screening Sequence	Definition Clinical HC	Diagnostic Criteria	Results	Quality
CHD, Coronary Artery Disease etc					
Waalen, 2002[79]	400 item questionnaire supplemented with medical record review to ensure ascertainment of all CHD events. Serum iron, TS and Ferritin values *HFE* C282Y and H63D mutations	NA	TS >55% in M and >45% in F, SF>250 µg/mL in M and >200 µg/mL in F were used to define elevated levels based on clinical criteria.	% C282Y/C282Y Males: All CHD 3/1798 = 0.17% 0 CHD 65/8540 = 0.76% Females: All CHD 3/1074 = 0.28% 0 CHD 65/9117 = 0.71%	Good
Rosenqvist 1989[76]	SF (ref range 25-200 µg); If 2x normal limit than serum iron and liver function tests; Liver biopsy on those patients who have two determinations of SF values more than 2x the upper limit HLA-A, -B tissue typing was performed	NA	NA	6 patients had increased SF concentrations 1 was too sick for a liver biopsy (myeloma was the cause of >SF) 1 died before liver biopsy could be done (post-mortem exam liver -no iron overload) 4 Liver biopsy 3/4 had iron overload (all three had AV block as reason for pacemaker) 3/232 (1.3%) of all pacemaker patients, and 3/149 = 2% of A-V block II-III patients, compared to 0.025% in a screening study comprising sera from 43,000 patients from the same region (Bjorn-Rasmussen,1985)	Fair

Appendix E Table 1 (continued). Studies of high-risk groups for C282Y homozygosity or HH.

Study Ref	Setting Time frame Country	Study Design	Sample	Risk Group Definition	Inclusion Exclusion	Population
Liver Disease Clinics						
Poullis, 2003[73] Moodie, 2002[74]	Patients attending a liver clinic at a teaching district general hospital in south London 1997-2001 London, England	Cross sectional data: Examining the value of routine TS testing of new liver clinic attendees over a 5 year period in detecting previously unrecognized cases of HH	667 out-patient referred for investigation of liver disease over 5 years Afro-Caribbean/African: not defined Asian: Majority originated from the Indian subcontinent, but also included two Chinese and four Iranians Mediterranean: families originated from Portugal and countries bordering the Mediterranean Northern European: not defined Celtic: defined as parents or grandparents from Cornwall, Wales, Scotland or Ireland	Outpatients referred to a liver clinic for investigation of liver disease.	Exclusion: previous HH diagnosis.	N=667 Age range 17-83 (median age 51) 68.6% European (38.4% Celtic, 30.2% other) 10.7% Asian, 9.7% Afro-Caribbean, 7.9% Mediterranean and 3.1% other Previous diagnoses: 28% hep C 6% primary biliary cirrhosis 4% hepatitis B N=349 Liver biopsy Previous diagnosis: 60% >30 units per week alcohol consumption, present or past history

Appendix E Table 1 (continued). Studies of high-risk groups for C282Y homozygosity or HH.

Study Ref	Initial Screening Sequence	Definition Clinical HC	Diagnostic Criteria	Results	Quality
Liver Disease Clinics					
Poullis, 2003[73] Moodie, 2002[74]	Non-fasting TS; those with TS >45% or a liver biopsy had *HFE* genotyping. Indications for biopsy included C282Y homozygosity, C282Y/H63D compound heterozogosity, elevated TS >60%, unexplained parenchymal liver disease and persistently abnormal liver function tests, and liver disease of known etiology needing staging or assessment of disease progression.	NA	TS cut offs	11/156 with TS >45% were C282Y/C282Y (7.1%) 1/349 liver patients with liver biopsy were C282Y/C282Y (0.03%) The prevalence of new HH cases in patients of European origin attending a liver clinic, detected by phenotypic screening over a 5-year period, was 2.8% (12/458 (Calc) (European only)).	Fair

Study Ref	Setting Time frame Country	Study Design	Sample	Risk Group Definition	Inclusion Exclusion	Population
Arthritis Studies						
Willis, 2002[78]	Specimens of arthritis patients from the DNA archive of the Norfolk Arthritis Register (NOAR) First diagnosed between 1989 and 1995 UK	Case Control: To determine the value of screening patients with inflammatory arthritis for HH-associated mutations in the *HFE* gene	Cases: Unselected inflammatory arthritis population collected by the Norfolk Arthritis Register and compared prevalence of the HH-associated *HFE* genotypes with a large sample from the normal populations Controls: 1000 individuals from the catchment area of the Norfolk and Norwich hospital, a large subset of the area covered by NOAR	People with inflammatory arthritis	**Cases:** **Inclusion**: Sequential DNA samples from patients for whom adequate DNA samples remained and who were first diagnosed between 1989 and 1995; more than one swollen joint lasting for more than 6 weeks. **Controls:** **Exclusions**: Hemochromatosis patients and people with foreign names	**Arthritis Populations:** N=1000 (average age 54 yr) **Controls:** 373 normal screening trial volunteers and 541 patients undergoing full blood counts Average age 54 .

Study Ref	Initial Screening Sequence	Definition Clinical HC	Diagnostic Criteria	Results			Quality
Arthritis Studies							
Willis, 2002[78]	*HFE* C282Y and H63D mutation testing	NA	NA		Arthritis Pts	Controls	Good
				Average age (yr)	54	54	
				C282Y homozygotes	5	5	
				Predicted C282Y homozygote freq.	1 in 287 (95% CI 190-403)	1 in 236 95% CI 170-335)	

Study Ref	Setting Time frame Country	Study Design	Sample	Risk Group Definition	Inclusion Exclusion	Population
Chronic Fatigue Syndrome						
Swinkels, 2002[77]	The department of general Internal medicine of the University Medical Centre St. Radbound, Nijmergen, a Dutch tertiary CFS referral Centre. 1992 Netherlands	Cross Sectional: To determine whether patients previously diagnosed as chronic fatigue syndrome (CFS) actually have primary hemochromatosis	NR	Patients fulfilling the criteria for CFS Had given permission to store serum for future CFS studies	NR	88 self-referred patients previously diagnosed with CFS Mean age 40 yrs (20-66) 23 males 65 females

TS-transferrin saturation; SF-serum ferritin; YY-C282Y/C282Y; NR-not reported; HH-hereditary hemochromatosis; M-male; F-female; TP-therapeutic phlebotomy; CFS-chronic fatigue syndrome.

Appendix E Table 1 (continued). Studies of high-risk groups for C282Y homozygosity or HH.

Study Ref	Initial Screening Sequence	Definition Clinical HC	Diagnostic Criteria	Results	Quality
Chronic Fatigue Syndrome					
Swinkels, 2002[77]	TS - Elevated if >40 for F and >45 for M. All pts that could be located with elevated TS N=15/19 asked to provide a new fasting blood sample for a second TS and SF. Genotyping if TS or SF levels were elevated (reference values for SF M 15-280 µg/L, pre and post menopausal F 6-80µg/L and 15-190µg/L respectively). N=6 with >TS N=2 with SF N=0 with >TS and SF	NA	NR	None of the 8 patients with >TS or >SF were either C282Y homozygotes nor compound C282Y/H63D heterozygotes.	Fair/ Poor

TS-transferrin saturation, SF-serum ferritin, YY-C282Y/C282Y, NR-not reported, HH-hereditary hemochromatosis, M-male, F-female; TP-therapeutic phlebotomy; CFS-chronic fatigue syndrome.

Appendix E Table 2. Studies excluded from key question 3.

Study Citation	Reason for exclusion
Adams PC, Agnew S. Alcoholism in hereditary hemochromatosis revisited: prevalence and clinical consequences among homozygous siblings. *Hepatology 23(4):724-7,* 1996.	Case series
Adams PC, Kertesz AE, Valberg LS. Clinical presentation of hemochromatosis: a changing scene. *American Journal of Medicine 90(4):445 -9,* 1991.	Case series
Adams PC, Kertesz AE, Valberg LS. Screening for hemochromatosis in children of homozygotes: prevalence and cost-effectiveness. *Hepatology.22(6):1720-7,* 1995.	<18 yrs included
Adams PC. Haemochromatosis: find them or forget about them? *European Journal of Gastroenterology & Hepatology 16(9):857-8,* 2004.	Editorial
Assy N, Adams PC. Predictive value of family history in diagnosis of hereditary hemochromatosis. *Digestive Diseases & Sciences.42(6):1312.-5,* 1997.	No HFE testing
Bacon BR, Olynyk JK, Brunt EM, Britton RS, Wolff RK. HFE genotype in patients with hemochromatosis and other liver diseases. *Annals of Internal Medicine 130(12):953 -62,* 1999.	Does not meet our definition of clinical hemochromatosis
Bassett ML, Halliday JW, Ferris RA, Powell LW. Diagnosis of hemochromatosis in young subjects: predictive accuracy of biochemical screening tests. *Gastroenterology 87(3):628 -33,* 1984.	Does not include primary results
Bassett ML, Halliday JW, Powell LW. Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis. *Hepatology 6(1):24-9,* 1986;-Feb.	Case series
Bhavnani M, Lloyd D, Bhattacharyya A, Marples J, Elton P, Worwood M. Screening for genetic haemochromatosis in blood samples with raised alanine aminotransferase. *Gut.46(5):707-10,* 2000.	Quality
Bonkovsky HL, Jawaid Q, Tortorelli K, LeClair P, Cobb J, Lambrecht RW et al. Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. *Journal of Hepatology 31(3):421-9,* 1999.	Does not meet our definition of clinical hemochromatosis
Bregman H, Gelfand MC, Winchester JF, Manz HJ, Knepshield JH, Schreiner GE. iron-overload-associated myopathy in patients on maintenance haemodialysis: a histocompatibility-linked disorder. *Lancet 2(8200):882 -5,* 1980.	Not the correction population
Brissot P, Moirand R, Jouanolle AM, Guyader D, Le Gall JY, Deugnier Y, David V. A genotypic study of 217 unrelated probands diagnosed as "genetic hemochromatosis" on "classical" phenotypic criteria. *Journal of Hepatology.30(4):588-93*, 1999.	Does not report relevant prevalence or risk measures
Campo S, Restuccia T, Villari D, Raffa G, Cucinotta D, Squadrito G, Pollicino T, Raimondo G. Analysis of haemochromatosis gene mutations in a population from the Mediterranean Basin. *Liver.21(4):233-6*, 2001.	Not the correction population

Appendix E Table 2 (continued). Studies excluded from key question 3.

Study Citation	Reason for exclusion
Cavanaugh JA, Wilson SR, Bassett ML. Genetic testing for HFE hemochromatosis in Australia: the value of testing relatives of simple heterozygotes. *Journal of Gastroenterology & Hepatology 17(7):800-3,* 2002.	Does not include primary results
Conte D, Manachino D, Colli A, Guala A, Aimo G, Andreoletti M, Corsetti M, Fraquelli M. Prevalence of genetic hemochromatosis in a cohort of Italian patients with diabetes mellitus. *Annals of Internal Medicine.128(5):370-3*, 1998.	Not the correction population
Dalury DF, Ewald FC, Christie MJ, Scott RD. Total knee arthroplasty in a group of patients less than 45 years of age. *Journal of Arthroplasty 10(5):598 -602,* 1995.	Does not report relevant prevalence or risk measures
Ellervik C, Mandrup-Poulsen T, Nordestgaard BG, Larsen LE, Appleyard M, Frandsen M et al. Prevalence of hereditary haemochromatosis in late-onset type 1 diabetes mellitus: a retrospective study. *Lancet 358(9291):1405-9,* 2001.	Not the correction population
Feller ER, Pont A, Wands JR, Carter EA, Foster G, Kourides IA et al. Familial hemochromatosis. Physiologic studies in the precirrhotic stage of the disease. *New England Journal of Medicine 296(25):1422 -6,* 1977.	Case series
Fiel MI, Schiano TD, Bodenheimer HC, Thung SN, King TW, Varma CR et al. Hereditary hemochromatosis in liver transplantation. *Liver Transplantation & Surgery 5(1):50-6,* 1999.	Does not report relevant prevalence or risk measures
Gleeson F, Ryan E, Barrett S, Crowe J. Clinical expression of haemochromatosis in Irish C282Y homozygotes identified through family screening. *European Journal of Gastroenterology & Hepatology 16(9):859-63,* 2004.	Does not report relevant prevalence or risk measures
Guyader D, Jacquelinet C, Moirand R, Turlin B, Mendler MH, Chaperon J et al. Noninvasive prediction of fibrosis in C282Y homozygous hemochromatosis. *Gastroenterology.115(4):929-936, 1998*	Not the correction population
Hultcrantz R, Gabrielsson N. Patients with persistent elevation of aminotransferases: investigation with ultrasonography, radionuclide imaging and liver biopsy. *Journal of Internal Medicine.233(1):7-12*, 1993.	Not relevant outcomes
Jeffrey GP, Adams PC. Pitfalls in the genetic diagnosis of hereditary hemochromatosis. *Genetic Testing.4(2):143-6, 2000.*	Editorial
Jordan JM. Arthritis in hemochromatosis or iron storage disease. *Current Opinion in Rheumatology 16(1):62-6,* 2004.	Review article
Jorquera F, Dominguez A, Diaz-Golpe V, Espinel J, Munoz F, Herrera A et al. C282Y and H63D mutations of the haemochromatosis gene in patients with iron overload. *Revista Espanola de Enfermedades Digestivas 93(5):293-302,* 2001.	Does not report relevant prevalence or risk measures
Koefoed P, Dalhoff K, Dissing J, Kramer I, Milman N, Pedersen P, Simonsen K, Tygstrup N, Nielsen FC. HFE mutations and hemochromatosis in Danish patients admitted for HFE genotyping. *Scandinavian Journal of Clinical & Laboratory Investigation.62(7):527-35*, 2002.	Quality

Appendix E Table 2 (continued). Studies excluded from key question 3.

Study Citation	Reason for exclusion
Krawczak M, Cooper DN, Schmidtke J. Estimating the efficacy and efficiency of cascade genetic screening. *American Journal of Human Genetics 69(2):361-70,* 2001.	Does not include primary results
Li J, Zhu Y, Singal DP. HFE gene mutations in patients with rheumatoid arthritis. *Journal of Rheumatology.27(9):2074.-7,* 2000.	Quality
MathewsJL, Williams HJ. Arthritis in hereditary hemochromatosis. *Arthritis & Rheumatism.30(10):1137-41,* 1987.	Not HFE
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. *Canadian Journal of Cardiology.14(2):215-20*, 1998.	Quality
Nelson RL, Persky V, Davis F, Becker E. Risk of disease in siblings of patients with hereditary hemochromatosis. *Digestion.64(2):120-4, 2001.*	Quality
Olynyk J, Hall P, Ahern M, Kwiatek R, Mackinnon M. Screening for genetic haemochromatosis in a rheumatology clinic. *Australian & New Zealand Journal of Medicine.24(1):22-5,* 1994.	Quality
Panajotopoulos N, Piperno A, Conte D, Mandelli C, Cesana M, Mercuriali F, Fiorelli G, Bianchi PA, Fargion D. HLA typing in 67 Italian patients with idiopathic hemochromatosis and their relatives. *Tissue Antigens.33(4):431-6*, 1989.	Not the correction population
Peterlin B, Globocnik PM, Makuc J, Hawlina M, Petrovic D. A hemochromatosis-causing mutation C282Y is a risk factor for proliferative diabetic retinopathy in Caucasians with type 2 diabetes. *Journal of Human Genetics 48(12):646-9,* 2003.	Not the correction population
Piperno A, D'Alba R, Fargion S, Roffi L, Sampietro M, Parma S et al. Liver iron concentration in chronic viral hepatitis: a study of 98 patients. *European Journal of Gastroenterology & Hepatology 7(12):1203 -8,* 1995.	Not the correction population
Rasmussen ML, Folsom AR, Catellier DJ, Tsai MY, Garg U, Eckfeldt JH. A prospective study of coronary heart disease and the hemochromatosis gene (HFE) C282Y mutation: the Atherosclerosis Risk in Communities (ARIC) study. *Atherosclerosis.154(3):739-46,* 2001.	Does not report relevant prevelance or risk measures
Roberts AG, Whatley SD, Morgan RR, Worwood M, Elder GH. Increased frequency of the haemochromatosis Cys282Tyr mutation in sporadic porphyria cutanea tarda. *Lancet 349(9048):321-3,* 1997.	Does not meet our definition of clinical hemochromatosis
Sampietro M, Piperno A, Lupica L, Arosio C, Vergani A, Corbetta N et al. High prevalence of the His63Asp HFE mutation in Italian patients with porphyria cutanea tarda. *Hepatology 27(1):181-4,* 1998.	Does not meet our definition of clinical hemochromatosis
Schmid H, Struppler C, Braun GS, Kellner W, Kellner H. Ankle and hindfoot arthropathy in hereditary hemochromatosis. *Journal of Rheumatology 30(1):196-9,* 2003.	Not the correction population

Appendix E Table 2 (continued). Studies excluded from key question 3.

Study Citation	Reason for exclusion
Sham RL, Raubertas RF, Braggins C, Cappuccio J, Gallagher M, Phatak PD. Asymptomatic hemochromatosis subjects: genotypic and phenotypic profiles. *Blood 96(12):3707 -11,* 2000.	Not the correction population
Shoaf EH, Jr. Hemochromatosis discovered through blood donor screening for alanine aminotransferase. *North Carolina Medical Journal 51(9):443-5,* 1990.	Case report
Siezenga MA, Rasp E, Wijermans PW. Testing families with HFE-related hereditary haemochromatosis. *Netherlands Journal of Medicine.62(5):156-9, 2004.*	Case report
Simon M, Alexandre JL, Bourel M, Le Marec B, Scordia C. Heredity of idiopathic haemochromatosis: a study of 106 families. *Clinical Genetics.11(5):327-41*, 1977.	Quality
Tannapfel A, Stolzel U, Kostler E, Melz S, Richter M, Keim V et al. C282Y and H63D mutation of the hemochromatosis gene in German porphyria cutanea tarda patients. *Virchows Archiv 439(1):1-5,* 2001.	Does not meet our definition of clinical hemochromatosis
Timms AE, Sathananthan R, Bradbury L, Athanasou NA, Wordsworth BP, Brown MA. Genetic testing for haemochromatosis in patients with chondrocalcinosis. *Annals of the Rheumatic Diseases.61(8):745-7,* 2002.	Quality