Digestive Diseases and Sciences, Vol. 49, No. 3 (March 2004), pp. 444–449 (© 2004)

Screening for Hemochromatosis in Turkey

HAKAN BOZKAYA, Assoc Prof Dr,* MEHMET BEKTAS, MD,* OLGA METIN, MD,† OZLEM ERKAN,‡ DICLE IBRAHIMOGLU, MD,† KLARA DALVA, MD,§ FILIZ AKBIYIK, MD,¶ SELIM GUREL, Assoc Prof Dr,|| ABDURRAHMAN MITHAT BOZDAYI, Assoc Prof Dr,‡ CEMAL AKAY, Assist Prof Dr,** CIHAN YURDAYDIN, Prof Dr,* ONDER ASLAN, Assoc Prof Dr, and OZDEN UZUNALIMOGLU, Prof Dr§

In this study we screened 3060 consecutive blood donors for an unbound iron-binding capacity level of <28 μM and then performed HFE mutation analysis in these subjects. Sixty-five of the 75 subjects with a low initial unbound iron-binding capacity (all had normal ferritin levels) came back and only 5 (8%) had a low fasting unbound iron-binding capacity. Mutational analysis revealed H63D heterozygosity in two of five subjects. Four of five subjects had liver biopsy indication and none had increased liver iron. HFE genotyping of 60 subjects with a low initial but normal fasting unbound iron-binding capacity revealed heterozygote H63D in seven (11.6%). No allelic variant of position 282 or 63 was found in three previously diagnosed patients with hereditary hemochromatosis. In conclusion, full phenotypic expression of hereditary hemochromatosis is very rare in Turkey. The absence of HFE mutations in three patients with hereditary hemochromatosis suggests that hereditary hemochromatosis in Turkey occurs without common HFE mutations.

KEY WORDS: hemochromatosis; screening; HFE mutations; blood donors.

Hereditary hemochromatosis (HH) is a common autosomal recessive disorder in the Caucasian population. Its frequency has been reported to be 0.3–0.5% in serological iron studies (1–7). It is more frequent in northern Europeans, particularly individuals of Nordic and Celtic ancetry, with a gene frequency of 1 in 200 (8–12). The responsible gene, HFE, is located on the short arm of chromosome 6 (13). A homozygote cysteine-to tyrosine substitution at position 282 (C282Y) in HFE gene has been found to be responsible in more than 90% of these patients (14–17). The role of a second mutation, a histidine-to-aspartic acid substitution at position 63 (H63D), in disease expression is less well understood. Compound heterozigosity of C282Y and H63D is reponsible for disease expression in 2–5% of cases. Although homozygote H63D mutation seems to be associated with iron overload, its role in fully expressed disease is less well understood (10, 18, 19). Despite the high frequency of homozygote C282Y in HH patients, data on its clinical penetrance are controversial. In some population-based studies, genotypic and phenotypic presentations show perfect correlation and most of the subjects carrying this mutation have clinically manifested iron overload (10). In contrast, some other population-based studies show that no more than half of the subjects with this mutation have phenotypically expressed disease (11, 20, 21).

The reported frequency of C282Y mutation in HH patients differs from one geographical area to another, while H63D mutation is observed worldwide (12, 18). Different geographical areas reflect different ethnic origins of subjects. Compared to North American and North European patients of Nordic and Celtic origin, the frequency of C282Y mutation is reported to be low in African American and southern European HH patients (22–26). Furthermore, HH can occur without pathogenic HFE mutations (27). In population screenings for HFE mutations, C282Y was found to be absent in Asians, Africans, and indigenous

Manuscript received November 12, 2002; accepted January 16, 2004.

From the Departments of *Gastroenterology; †Internal Medicine, and §Haematology, Ankara University Medical School, Ankara, ‡Institute of Hepatology, Ankara University, Ankara, ¶Department of Biochemistry, Hacettepe University, Ankara, ||Department of Gastroenterology, Uludag University, Bursa, and **Department of Toxicology, Gulhane Medical School, Ankara Turkey.

Address for reprint requests: Prof. Dr. Hakan Bozkaya, Ankara University, Gastroenteroloji Departmani, Cebeci Kampusu, 06100 Dikimevi, Ankara, Turkey; bozkaya@medicine.ankara.edu.tr.

Australasians, while the H63D mutation had a worldwide distribution (12, 20, 28). However, this informations is mainly based on screening studies from West Europe or America and Australia in which subjects of different ethnic origins represented a small proportion of those screened. Thus, there is still a need for region/country-based population screening studies in eastern Europe and Asian countries to understand the true prevalance and phenotypic–genotypic correlations of the disease in these regions.

High frequency and increased disease associated morbidity and mortality of HH in Western populations resulted in the development of cost-effective population screening strategies (29, 30). Indeed, screening for hemochromatosis has been found to be cost-effective in the medical care setting and in the general population in western Europe, North America, and Australia (6, 31–35). Today, phenotypic screening by transferin saturation (TS) or unbound iron-binding capacity (UIBC) followed by mutational analysis in subjects with iron overload is the preferred strategy in population screening studies (29, 36, 37).

To date, no study has addressed population screening for HH in Turkey. Thus the aim of this study was to screen healthy blood donors for iron overload and then perform mutational analysis for common HFE mutations in these subjects.

MATERIALS AND METHODS

Screening Strategy. Over a period of 4 months, 3060 consecutive healthy blood donors (2780 male, 280 female) over the age of 20 years (range, 20–64 years; mean, 32 years) who attended Ankara University Ibn-i Sina Hospital blood bank for blood donation were screened. All subjects were informed about the details of the study and informed consent was obtained from all donors. The study was approved by the ethics committee of Ankara University Medical School. Donors' age, gender, address, and telephone number were recorded. Serologies for hepatitis B, hepatitis C, and HIV were all noted. Serum samples obtained from each donor were frozen at -80°C until UIBC determinations and a UIBC value of less than 28 μM was accepted as the screening cutoff point for iron overload. The next step was to invite the subjects with a low UIBC level in the initial test for fasting UIBC and TS determinations. At the same time, whole blood for mutational analysis was collected. Patients with a UIBC value of less than 28 μM or with a TS level greater than 45% were subjected to HFE mutation analysis. If a patient was found to be a homozygote for C282Y mutation or displayed compound heterozygosity, he was invited for a liver biopsy for quantitative liver iron assessment. If these HFE mutations were absent, liver iron was still determined when a patient had a high ALT ($>$37 IU/L) and/or ferritin ($>$300 μg/L in men and $>$200 μg/L in women) level; if this was not the case, patients were followed with repeated UIBC testing. This strategy is summarized in Figure 1.

In addition, two brothers with previously diagnosed hemochromatosis (38) and their sister and both parents and another previously diagnosed hemochromatosis patient and his two brothers and mother were analyzed for HFE mutations.

UIBC Determination. UIBC was determined by a colorimetric method according to the manufacturer's instructions (Randox Laboratories, Ltd., Antrim, UK) using Alciaon 300i (Abbott) analyzer for the assay.

TS Determination. TS was calculated on the basis of automated colorimetric determination of serum iron and total iron-binding capacity (Sigma Diagnostics, USA).

Ferritin Measurement. Ferritin level was measured with an Immulite ferritin kit according to the manufacturer's instructions (Diagnostic Products Corp., Los Angeles, CA, USA). The assays were performed using an Immulite analyzer.

HFE Mutation Analysis. After preparation of genomic DNA from whole blood, mutational analysis was performed by restriction fragment length polymorphism analysis of PCR-amplified genomic DNA as previously described (39).

Liver Iron Determinations. Qualitative iron determination was performed by Perls' Prussian blue staining. Quantitative iron measurement (μg/g dry weight) was done by atomic absorption spectrophotometry (Varian 30/40, Australia).

RESULTS

Seventy-five (67 male, 8 female; mean age, 31.6 years; range, 20–60 years) of 3060 subjects had a UIBC value of $<$28 μM. None of these 75 subjects had increased serum ferritin levels. Sixty-five of them (87%) came back for fasting UIBC determination and for HFE genotyping and most had normal fasting UIBC levels. Only 5 of the 65 (8%) subjects had a UIBC level of less than 28 μM (Figure 2) and their clinical features are listed in Table 1. Three of 65 subjects, all with UIBC values of less than 28 μM, had an elevated TS value ($>$45%). Mutational analysis of these five subjects revealed no homozygote C282Y or compound heterozygosity, while two subjects had heterozygote H63D. Abdominal ultrasonographic examination showed increased paranchimal echogenisity in four of five subjects without any evidence of advanced liver disease/portal hypertension. One of them had a history of more than 40-g alcohol consumption per week. Apart from this subject, four of the five subjects had liver biopsy indication according to the protocol and all four accepted the liver biopsy. The subject with a history of alcohol abuse had normal ALT and ferritin levels and repeat UIBC levels were higher than 28 μM. Indications for liver biopsy were mild ALT elevations in three subjects and a persistently low UIBC level in one. One subject with ALT elevation was also hepatitis B surface antigen (HBsAg)-positive and routine examination of hemotoxylin and eosin staining revealed periportal inflammation. Liver histology was consistent with steatosis in the remaining three cases. However, none of these four subjects had positive staining

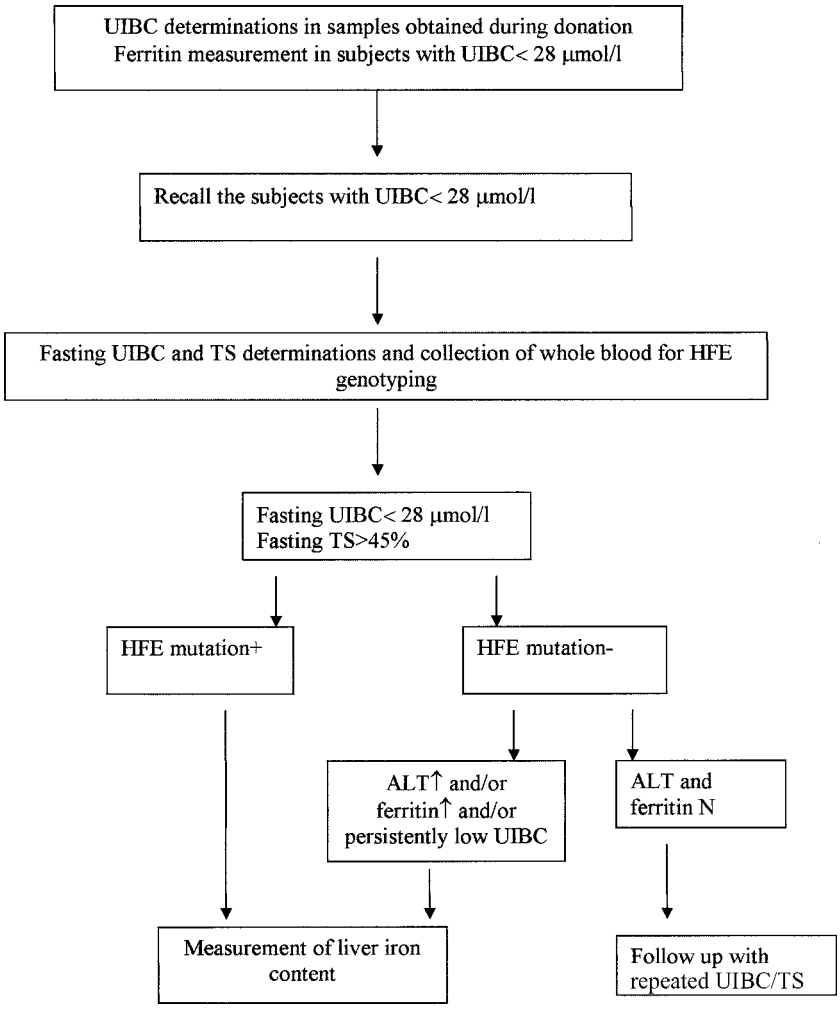

Fig 1. Schematic illustration of the hemochromatosis screening strategy used in this study.

for iron with Perls' Prussian blue and none had increased iron stores quantitatively measured by atomic absorption spectrophotometry.

HFE genotyping was extended to the remaining 60 subjects who had low UIBC levels on initial testing but normal levels in the fasting state. None of these subjects had homozygote C282Y or compound heterozygosity of C282Y and H63D. Seven of them (11.6%) were heterozygote for H63D.

Both brothers with a previous diagnosis of hemochromatosis had hyperpigmentation and hepatomegaly and one of them also had diabetes mellitus. Transferrin saturations were 78 and 67% and ferritin levels were 3551 and 3640 ng/ml. Other male patient also had diabetes mellitus, and at the time of the diagnosis, his transferrin saturation and ferritin level were 76% and 3507 mg/ml, respectively. None of the patients had thalassemia or any evidence of hemolytic anemia and all three patients consumed less than 20 g alcohol per day. They all were HLA antigen A3-positive and liver biopsies revealed grade 3 stainable iron deposition according to Scheuer *et al.* (40). HFE mutation analysis in the two brothers with genetic hemochromatosis and their sister and parents and in another patient and his two brothers and mother did not reveal any allelic variant at position 282 or 63.

DISCUSSION

The results of this study suggest that HH is very rare in the Turkish population. Not a single case of phenotypically expressed HH could be identified by screening over 3000 healthy blood donors. It is thus not surprising that hemochromatosis is worth publishing as a case report in Turkey (38). This study thus supports the evidence that HH is most prevalent in populations of northern European extraction (29). Although it is known that the disease is

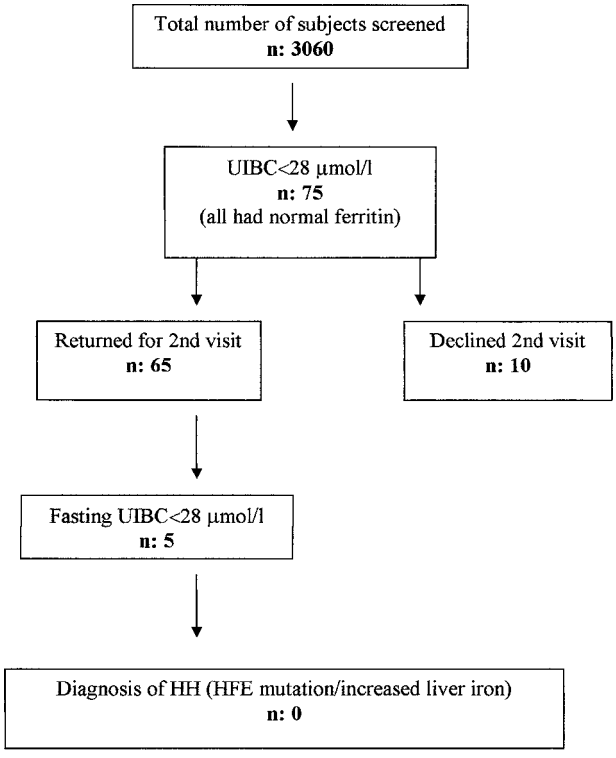

Fig 2. Schematic representation of the outcome of subjects screened in this study.

less frequent in southern European populations, data are lacking on its prevalance in eastern Europe. The finding of an absence of C282Y or H63D mutations in our three patients with previously documented diagnosis of HH and in their first-degree relatives suggests that in the rare cases of HH in Turkey, HH occurs without the common pathogenic mutations in the hemochromatosis gene, as has been described in a family cohort study from Italy (27).

We observed that iron overload is infrequent in the Turkish population. Low UIBC levels ($<28\ \mu$M) were detected in 75 of 3060 (2.4%) subjects during initial determination. Sixtyfive of these 75 subjects returned for fasting UIBC determination but only 5 (8%) were documented to have low fasting UIBC levels. This is in line with previous observations. In one of the largest studies reported, only 13% of the subjects with elevated TS in initial testing were found to have elevated fasting TS (3). These findings underline the importance of fasting determinations of TS or UIBC to avoid false-positive results. Furthermore, persistence of TS elevation rather than documantation of iron overload on one occasion seems to be more important. A recent study reported that persistent elevation of TS was infrequent in the absence of C282Y mutation over a 4-year period after initial documentation of elevated TS (10).

Nevertheless, genotyping of not only the 5 subjects with low fasting UIBC but also the 60 subjects with initial low UIBC levels was undertaken. None of these 65 subjects had homozygote C282 or compound heterozygosity of C282 and H63D. In 7 of the 65 subjects, a heterozygote state for H63D was found and 2 of them had low fasting UIBC levels. Apart from homoziygote C282 and compound heterozygosity, the importance of homozygote H63D and heterozygote state for H63D or C282Y is less well understood. Some reports suggested that homozygote H63D is responsible for some phenotypically expressed HH (41), but the role of this mutation in the pathogenesis of the disease may still be considered uncertain (13, 29). There is also evidence suggesting that H63D or C282 heterozygotes have higher TS and ferritin and lower UIBC levels compared to wild-type 282 or 63 (8, 20, 21). A recent study on expression patterns of HFE mRNAs in peripheral blood cells from heterozygotes suggested that mutated transcripts can be predominant in H63D and that this mutation may play a role in disease expression, especially when associated with environmental or host factors (42). Further studies on large number of subjects carrying H63D allele are necessary to understand the importance of this mutation in iron overload in the Turkish population.

The possible reasons for low UIBC levels in five subjects are alcohol consumption in one, nonalcoholic fatty liver in three, and chronic hepatitis B infection in one case. Despite the low UIBC levels in these subjects, the iron content of the liver of four subjects in whom liver biopsy

TABLE 1. FEATURES OF SUBJECTS WITH LOW FASTING UIBC LEVELS

Subject No.	*Sex*	*Age (yr)*	*UIBC (μm)*	*Ferritin (μg/l)*	*ALT (0–37 IU/L)*	*HFE position**		*Liver biopsy†*
						282	*63*	
1	Male	32	14.3	89.8	23	w/w	w/w	NP
2	Male	24	8.9	91.4	40	w/w	w/w	+
3	Male	24	10.5	120	12	w/w	w/w	+
4	Male	30	18.5	58.5	39	w/w	w/w	+
5	Male	39	20	276	56	w/w	w/w	+

*w/w, wild type for both alleles; m/w, mutant for one allele (heterozygote). †NP, not performed; +, performed.

was available was not found to be increased. However, follow-up periods of these subjects are short and long-term follow-up is necessary to see whether the low UIBC level persists. They are now being followed up regularly at our hepatology unit, with periodic determinations of UIBC, TS, and ferritin levels.

In summary, full phenotypic expression of hereditary hemochromatosis is very rare in Turkey. The absence of HFE mutations in three patients with a diagnosis of HH suggests that HH in Turkey may occur without pathogenic mutations in the HFE gene. The results of our study further suggest that population screening for HH is not indicated in Turkey.

ACKNOWLEDGMENT

This project (No. 2001-08-09-069) was supported by Ankara University Research Foundation.

REFERENCES

1. Olson KS, Ritter B, Rosen U, Heedman PA, Staugard F: Prevalence of iron overload in Central Sweden. Acta Med Scand 213:145–150, 1983
2. Olson KS, Eriksson K, Ritter B, Heedman PA: Screening for iron overload using transferrin saturation. Acta Med Scand 215:105–112, 1984
3. Edwards CQ, Griffen LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP: Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. N Engl J Med 318:1355–1362, 1988
4. Leggett BA, Halliday JW, Brown NN, Bryant S, Powell LW: Prevalence of haemochromatosis amongst asymptomatic Australians. Br J Haematol 74:525–530, 1990
5. Wiggers P, Dalhoj J, Kier H, Ring-Larsen H, Petersen PH, Blaabjerg O: Screening for haemochromatosis: prevalance among Danish blood donors. J Intern Med 230:265–270, 1991
6. Baer DM, Simons JL, Stables RL, Rumore GJ, Morton CJ: Hemochromatosis screening in asymptomatic ambulatory men 30 years of age and older. Am J Med 98:464–468, 1995
7. Smith BN, Kantrowitz W, Grace ND, Greenberg MS, Patton TJ, Ookubo R, Sorger K, Semeraro JG, Doyle JR, Cooper AG, Kamat BR, Maregni LM, Rand WM: Prevalence of hereditary hemochromatosis in a Massachusetts corporation: is Celtic origin a risk factor? Hepatology 25:1439–1446, 1997
8. Burt MJ, George PM, Upton JD, Collett JA, Frampton CM, Chapman TM, Walmsly TA, Chapman BA: The significance of haemochromatosis gene mutations in the general population: implications for screening. Gut 43:830–836, 1998
9. Bassett ML, Halliday JW, Bryant S, Dent O, Powell LW: Screening for hemochromatosis. Ann NY Acad Sci 526:274–278, 1988
10. Olynyk JK, Cullen DJ, Aquilia S, Rossi E Summerville L, Powell LW: A population based study of clinical expression of the hemochromatosis gene. N Engl J Med 341:718–724, 1999
11. Adams PC, Kertesz AE, McLaren CE, Barr R, Bamford A, Chakrabarti S: Population screening for hemochromatosis. A comparison of unbound iron binding capacity, transferrin saturation, and C282Y genotyping in 5211 voluntary blood donors. Hepatology 31:1160–1164, 2000
12. Merryweather-Clarke AT, Pointon JJ, Sherman JD, Robson KJ: Global prevalence of putative hemochromatosis mutations. J Med Genet 34:275–278, 1997
13. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R Jr, Ellis MC, Fullan A, Hinton LM, Jones NL, Kimmel BE, Kronmal GS, Lauer P, Lee VK, Loeb DB, Mapa FA, McClelland E, Meyer NC, Mintier GA, Moeller N, Moore T, Morikang E, Wolf RK: A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 13:399–408, 1996
14. Jazwinska EC, Cullen LM, Busfield F, Pyper WR, Webb SI, Powell LW, Morris CP, Walsh TP: Haemochromatsosis and HLA-H. Nat Genet 14:249–251, 1996
15. Jouanolle AM, Gandon G, Jezequel P, Blayau M, Campion ML, Yaounq J,. Mosser J, Fergelot P, Chauvel B, Bouric P, Carn G, Andrieux N, Gicquel I, Le Gall JY, David V: Haemochromatosis HLA-H. Nat Genet 14:251–252, 1996
16. The UK Haemochromatosis Consortium: A simple genetic test identifies 90% of UK patients with haemochromatosis. Gut 41:841–844, 1997
17. Adams PC, Chakrabarti S: Genotypic/phenotypic correlations in genetic hemochromatosis: evaluation of diagnostic criteria. Gastroenterology 114:319–323, 1998
18. Burke W, Thomson E, Khoury MJ, McDonnell SM, Pres N, Adams PC, Barton JC, Beutler E, Brittenham G, Buchanan A, Clayton EW, Cogswell ME, Meslin EM, Motulsky AG, Powell LW, Sigal E, Wilfond BS, Collins FS: Hereditary hemochromatosis: gene discovery and implications for population-based screening. JAMA 280:172–178, 1998
19. Moirand R, Jouanolle AM, Brissot P, Le Gall J, Deugnier Y, David V: Phenotypic expression of HFE mutations: a French study of 1110 unrelated iron-overloaded patients and relatives. Gastroenterology 116:372–377, 1999
20. Beutler E, Felitti V, Gelbart T, Ho N: The effect of HFE genotypes on measurements of iron overload in patients attending a health appraisal clinic. Ann of Intern Med 133:329–337, 2000
21. Jackson HA, Carter K, Darke C, Guttridge MG, Ravine D, Hutton RD, Napier JA, Worwood M: HFE mutations, iron deficiency and overload in 10 500 blood donors. Br J Haematol 114:474–484, 2001
22. Wurapa RK, Gordeuk VR, Brittenham GM, Khiyami A, Schecter GP, Edwards CO: primary iron overload in African Americans. Am J Med 101:9–18, 1996
23. Monaghan KG, Rybicki BA, Shurafa M, Feldman GL: Mutation analysis of the HFE gene associated with hereditary hemochromatosis in Africans Americans. Am J Hematol 58:213–217, 1998
24. Gordeuk VR, McLaren CE, Looker AC, Hasselblad V, Brittenham GM: Distribution of transferrin saturations in the African-American population. Blood 91:2175–2179, 1998
25. Carella M, D'Ambrossio L, Totara A, Grifa A, Valentino MA, Piperno A, Girelli D, Roetto A, Franco B, Gasparini P, Camaschella C: Mutation analysis of HLA-H gene in Italian hemochromatosis patients. Am Hum Genet 60:828–832, 1997
26. Piperno A, Sampietro M, Pietrangelo A, Arosio C, Lupica L, Montosi G, Vergani A, Fraquelli M, Girelli D, Pasquero P, Roetto A, Gasparini P, Fargion S, Conte D, Camaschella C: Heterogenity of hemochromatosis in Italy. Gastroenterology 114:996–1002, 1998
27. Pietrangelo A, Montosi G, Totaro A, Garyti C, Conte D, Cassanelli S, Fraquelli M, Sardini C, Vasta F, Gasparini P: Hereditary hemochromatosis in adults without pathogenic mutations in the hemochromatosis gene. N Eng J Med 341:725–732, 1999

28. Cullen LM, Summreville L, Glassick TV Crawford DH, Powell LW, Jazwinska EC: Neonatal screening for the haemochromatosis defect. Blood 90:4236–4237, 1997
29. Powell LW, Subramaniam VN, Yapp TR: Haemochromatosis in the new millennium. J Hepatol 32 (Supp 1):48–62, 1999
30. Yang Q, McDonnell SM, Khoury MJ, Cono J, Parrish RG: Hemochromatosis-associated mortality in the United states from 1979 to 1992: an analysis of multiple-cause mortality data. Ann Intern Med 129:946–953, 1998
31. 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 107:453–459, 1994
32. Phatak PD, Guzman G, Woll JE, Robeson A, Phelps CE: Cost-effectiveness of screening for hereditary hemochromatosis. Arch Intern Med 154:769–776, 1994
33. Adams PC, Gregor JC, Kertesz AE, Valberg LS: Screening blood donors for hereditary hemochromatosis: decision analysis based on a 30 year database. Gastroenterology 109: 177–188, 1995
34. Adams PC, Valberg LS: Screening blood donors for hereditary hemochromatosis: decision analysis model comparing genotyping to phenotyping. Am J Gastroenterol 94:1593–1600, 1999
35. Basset ML, Leggett BA, Halliday JW, Webb S; Powell LW: Analysis of the cost of population screening for hemochromatosis using biochemical and genetic markers. J Hepatol 27:517–524, 1997
36. Witte DL, Crosby WH, Edwards CQ, Fairbanks VF, Mitros FA: Practice guideline development task force of the College of American Pathologists. Hereditary hemochromatosis. Clin Chim Acta 245:139–200, 1996
37. Adams PC: Population screening for haemochromatosis. Gut 46:301–303, 2000
38. Gurel S: Genetic hemochromatosis: a report of two cases. Turk J Gastroenterol 9:385–387, 1998
39. Jouanolle AM, Fergelot P, Gandom G, Yaouanq J, Le Gall JY, David V: A candidate gene for hemochromatosis: frequency of the C282Y and H63D. Hum Genet 100:544–547, 1997
40. Scheuer PJ, Williams R, Muir AR: Hepatic pathology in relatives of patients with haemochromatosis. J Pathol Bacteriol 84:53–64, 1962
41. Aguilar-Martinez P, Bismuth M, Picot MC, Thelcide C, Paageaux GP, Blanc F, Blanc P, Schved JF, Larrey D: Variable phenotypic presentation of iron overload in H63D homozygotes: are genetic modifiers the cause? Gut 48:836–842, 2001
42. Rosmorduc O, Poupon R, Nion I, Wendum D, Feder J, Bereziat G, Hermelin B: Differential HFE allele expression in hemochromatois heterozygotes. Gastroenterology 119:1075–1086, 2000