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Hemochromatosis gene and nonalcoholic fatty liver disease: a systematic review and meta-analysis

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Abstract

Background and aims—Previous studies examining the relationship between the C282Y and H63D *HFE* mutations and presence of nonalcoholic fatty liver disease (NAFLD) have yielded conflicting results. The goal of this study was to systematically evaluate and summarize data on the association between these two variants and the presence of NAFLD.

Methods—The authors searched EMBASE and PUBMED from August 1, 1996 to August 12, 2010. Two investigators independently conducted data abstraction. Ethnic specific weighted prevalence was calculated, and pooled odds ratios were estimated using random effects model.

Results—From 2,542 references, the authors included 16 case-control studies and 14 case-only studies, or 2,610 cases and 7,298 controls. The majority of the studies came from Caucasian populations (2,287 cases and 4,275 controls). The weighted prevalence of *HFE* mutations in cases was comparable to controls. The meta-analysis was restricted to Caucasians only because the small sample size of non Caucasian participants. The pooled odds ratio for the presence of any *HFE* genetic variant in cases was 1.03 (95% CI: 0.90, 1.17; I^2 : 65.8%, 95% CI: 38.5, 81.0). The presence of other genotypes, and secondary analyses yielded similar non significant findings.

Conclusion—Our systematic review does not support an association between the *HFE* genetic variants and the presence of NAFLD.

Nonalcoholic fatty liver disease (NAFLD), the most common chronic liver disease [1], represents a wide spectrum of disease characterized by the presence hepatic steatosis in the

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absence of significant alcohol consumption or other causes of liver disease [2, 3] The pathogenic processes leading to steatosis, steatohepatitis and fibrosis are multifactorial and involve both environmental and genetic factors [4, 5]. Iron overload has been reported in NAFLD and could contribute to its pathogenesis and progression. Iron overload influences lipid [6, 7] and glucose metabolism [8], and thus may lead to insulin resistance, a key factor in the pathogenesis of NAFLD. Furthermore, iron excess may also contribute to progression of NAFLD thru oxidative stress and fibrogenesis within the liver [9, 10].

Feder et al. identified in 1996 the cysteine-to-tyrosine substitution at the amino acid 282 (C282Y), and the histidine-to-aspartate change at the amino acid 63 (H63D) variants of the *HFE* gene (OMIM 235200) [11]. These variants are responsible for the majority of cases of hereditary hemochromatosis, and are very common in Caucasians (6.2% and 15.1%, respectively) [12]. Compared to other genotypes, C282Y homozygotes are at greatest risk of iron overload, followed by compound heterozygotes with one C282Y mutant allele and one H63D mutant allele; on the contrary, simple heterozygotes for both C282Y and H63D may also develop increased serum iron markers, but to a lesser extent [13–16]. Since NAFLD and *HFE* mutations are common, many authors studied the association between these two conditions and found conflicting results. Inadequate sample size, referral and ascertainment biases, different ethnic background, lack of mediators' adjustment, or publication bias could explain such inconsistencies. A wide scope meta-analysis published suggested a positive association for some genotypes with NAFLD [17]; in this paper, however, we performed a meta-analyses focused in NAFLD with updated literature search.

The main goal of this study was to systematically review the available evidence examining the association between the common *HFE* genetic variants (C282Y and H63D) and NAFLD. We specifically studied whether the *HFE* mutations were enriched in NAFLD patients compared to non diseased controls; we also studied the association between C282Y homozygotes and C282Y/H63D because these are the most iron loading genotypes. We hypothesized that the *HFE* genetic variants would be more frequent in NAFLD patients compared to controls. A positive association would suggest that *HFE* could identify patients at risk for NAFLD and, that iron depletion by phlebotomy could be explored as a safe and easy therapy for NAFLD-*HFE* (+) patients.

MATERIAL AND METHODS

Search strategy and study selection

We aimed to identify all studies that assessed the association of the C282Y and H63D genetic variants and NAFLD using a previously defined protocol [18]. We searched PUBMED and EMBASE for the period between August 1st, 1996 (when *HFE* was firstly described) [11] and August 12th, 2010 using free text and subheadings with explosion (MeSH and Emtree terms) and without language restriction (Appendix A and Figure S1). In addition, we reviewed the reference lists of relevant original papers and review articles. We excluded papers without original data, animal, or in vitro studies. We also excluded those papers whose inclusion criteria required carrier status for *HFE* mutations [19]. Lastly, we also excluded studies that reported only extreme phenotypes (hepatocellular carcinoma and cirrhosis) since their inclusion may introduce phenotypic heterogeneity.

Given that some studies in Caucasian populations did not have a control group, we performed secondary analyses imputing a control group from the non Hispanic Whites (NHW) of the Third National Health and Nutrition Examination Survey (NHANES III) to enrich the number of participants [12].

Data abstraction

Two investigators (R.H. and E.Y) independently reviewed the search results to determine study eligibility and to perform data abstraction. Discrepancies were resolved by group consensus. For each selected publication we abstracted key study characteristics and genotype frequencies. When the genotypes of interest were not directly shown, we tried first to calculate them from the published data [20, 21], or, if not possible, we contacted the authors. For the quality of the reporting, we used a modified STREGA checklist (Appendix B) [22], and MOOSE recommendations [23].

Statistical methods

Since both C282Y and H63D are functional variants [24, 25], confounding by population stratification may be less of a concern in this study. That is, both mutations have the same effect across different ethnicities. Since the *HFE* genotype distribution [26, 27] and NAFLD [28] prevalence varies between ethnicities, we made the *a priori* decision to stratify our analyses by ethnicity. Since the majority of the papers did not explicitly report the ethnicity/race of the participants, we decided to consider 'Caucasian' those studies which reported a majority of Caucasians (e.g. least 70%), or that were conducted in countries whose majority is mainly Caucasian (i.e. Europe, Australia and North America).

For those studies that included a control group, we computed the odds ratios (OR) and 95% confidence intervals for NAFLD comparing the presence of any *HFE* mutation (C282Y/C282Y, C282Y/H63D, C282Y/WT, H63D/H63D, H63D/WT) to non diseased controls. We also obtained pooled OR for all possible genotypes, and created different subgroups such as those genotypes traditionally associated with iron overload (C282Y/C282Y and C282Y/H63D), or mixed heterozygotes (C282Y/H63D, C282Y/WT, and H63D/WT)[17].

In order to combine those studies without control group, we imputed the genotype frequencies from the non Hispanic Whites population in a representative sample of the U.S. population [12]. We used the U.S. weighted frequencies reported by Steinberg et al. (C282Y/C282Y: 0.30%; C282Y/H63D: 2.35%; H63D/H63D: 2.15%; C282Y/WT: 9.54%; H63D/WT, 23.55%; and, WT/WT: 62.10%. [12]

We considered the reference group those individuals wild type at both loci (WT/WT). We used the Cochran's Q-test with a significance level of $p = 0.10$ to qualitatively assess statistical heterogeneity [29], and the I^2 statistic with the 95% confidence intervals to quantify the proportion of total variability in point estimates that can be attributed to heterogeneity [30]. To account for possible statistical heterogeneity, we adopted the inverse variance method with random effects model to calculate the ethnic specific pooled odds ratios [30–32]. If statistical heterogeneity was present, we analyzed the impact on the interpretation of pooled OR by stratifying on clinically relevant characteristics. We decided to provide a pooled estimate when, despite statistical heterogeneity, the inference remained unchanged. We assessed also the presence of publication bias by the Harbord regression test for funnel-plot asymmetry [33]. All analyses were done using the updated commands *metan*, *metabias*, and *metainf* available in STATA 9.2 (College Station, Tx). A portion of these analyses were presented at the 60th American Association for the Study of Liver Disease Meeting [34].

RESULTS

We identified 2,542 unique references (2,272 in EMBASE; 1,602 in MEDLINE), of which 2,359 did not meet our inclusion criteria. We excluded 153 studies due to lack of data on the *HFE* genotypes in NAFLD participants; whose liver disease was not defined as NAFLD; or whose participants were reported included in separate reports. A total of 16 case-control

studies [20, 21, 35–49] (Table 1) and 14 ‘case-only’ studies [50–63] (Table 2), or 2,610 cases and 7,298 controls were included for the current review.

Overall, twenty-two studies were originated in Europe, North America, or Australia [20, 21, 35, 37–40, 42–45, 47, 48, 50, 51, 54–60, 63], two studies in Brazil [49, 52] and Japan [61, 62], respectively and, one study each in Korea [36], Taiwan [41], India [53] and Turkey [46]. The quality of the reporting varied across the papers. Whereas the indication for the liver study, the NAFLD definition, and the outcome ascertainment among cases were usually reported, only nine studies reported the ethnicity of the participants [20, 36–38, 47, 49, 52, 58, 62, 63], three reported some quality control in the DNA collection [36, 37, 40], and three excluded C282Y homozygotes from the study [36, 42, 48, 58]. (Appendix C). The most common reason for investigation of possible NAFLD was elevation in liver enzymes and/or an “echogenic” liver on an ultrasound examination. The method for ascertainment of NAFLD cases was pathology in most studies, and most cases in such studies were nonalcoholic steatohepatitis (NASH), rather than simple steatosis. For definition of controls, seven used clinical and imaging criteria to rule out NAFLD [35, 36, 43, 44, 47–49], and others used blood donors [20, 39, 47], newborns [21, 45, 46] or did not describe the methodology [37, 38, 41, 42]. Specifically for the case-control studies, the majority of the participants had presumed Caucasian ethnicity (1,727 cases and 4,275 controls), three had iron overload as the criteria for inclusion [42, 44, 48], six used newborns or blood donors as controls [20, 21, 39, 45–47], and four studies used only ultrasound to define NAFLD [36, 40, 41, 44].

The internally weighted prevalence for the *HFE* genotypes in people with NAFLD is depicted in Figure S2. Among Caucasians, the prevalence of C282Y and H63D homozygotes was 1.01% (95% confidence interval, CI: 0.60, 1.41%) and 2.01% (95% CI: 1.44, 2.59%), respectively. The prevalence of compound heterozygotes, C282Y heterozygotes, and H63D heterozygotes were 1.66% (95% CI: 1.14, 2.19%), 10.76% (95% CI: 9.49%, 12.03%), and 16.70% (95% CI: 15.17, 18.23%), respectively, which are comparable to the control group and non-Hispanic White U.S. population [12]. In contrast, no C282Y mutation was present in non-Caucasian populations, which had H63D heterozygotes as the most common genotype after WT/WT.

The odds ratio for NAFLD among Caucasians was not significantly increased for those carrying *HFE* mutations (C282Y or H63D) compared to controls (OR: 1.03, 95% CI: 0.90, 1.17). Among non-Caucasians, there was a significant association (OR: 1.64; 95% CI: 1.20, 2.24), Figure. There was stronger evidence for statistical heterogeneity in the odds ratio between Caucasians (I^2 : 65.8%, 95% CI: 38.5, 81.0) to non-Caucasians (I^2 : 0%; 95% CI: 0, 85.1). Given the small number of studies in non Caucasian population, we restricted the following analyses among Caucasians only. We addressed statistical heterogeneity by studying different clinically relevant characteristics as previously described. Our inference of no association between the presence of any *HFE* mutation and NAFLD remained unchanged. Considering that previous authors encountered association with a combination of heterozygotes [17] we conducted similar analyses and found no association. Similar results were also found with a category that included both C282Y/C282Y and C282Y/H63D, the most penetrant (iron loading) genotypes. All the previous analyses were also stratified by clinical characteristics (Supplementary Table) and reached similar conclusions without statistical evidence for publication bias as shown by Harbord tests (Figure S3).

Finally, in order to include the genotypes of those 14 studies without control group, we imputed the genotype frequencies using the non-Hispanic white population and found that the presence of any *HFE* mutation was not associated with NAFLD (OR: 1.00, 95% CI: 0.87, 1.14). When considering different genotypes, only the presence of C282Y/C282Y was

associated with NAFLD (OR: 6.50, 95% CI: 2.30, 18.35), and other categories including C282Y homozygotes (Figure S4).

DISCUSSION

Our current systematic review and meta-analysis of 13 case-control studies did not find a significant association between the presence of *HFE* mutations and NAFLD in Caucasians. Specifically, we showed that the presence of iron overloading genotypes (C282Y/C282Y, C282Y/H63D) did not increase the risk of having NAFLD compared to controls. When we imputed controls from NHANES, we found similar inferences except from the presence of homozygosity. This may be explained by the presence of oversampling of cases with C282Y homozygosity among the case-only studies rather than a real association. In non Caucasian populations, our results based on three case-control studies suggest an excess of H63D mutants in NAFLD cases compared to controls.

In contrast to Ellervik et al [17], our analyses showed no association due, we believe, to three key factors. First, applying the criteria they described, two studies should not have been excluded [45, 49], and, among the studies included, Ellervik et al. did not abstract the studies with the greatest number of cases [37, 38, 47] in the meta-analysis. Secondly, weights used for meta-analysis appear to be inconsistent from the confidence intervals estimated by Ellervik and colleagues (Ellervik et al., Webfigures 6 and 9). In other words, using the abstracted study-specific odds ratios and 99% confidence intervals (Webfigures 6 and 9) we found incorrect weights and, consequently, inaccurate pooled odds ratios for the C282Y/C282Y genotypes and mixed heterozygotes, but not for the H63D/H63D genotype. Finally, two of the studies abstracted by Ellervik et al. did not genotype H63D in all individuals and this fact was not taken into account in the prior meta-analysis [20, 21]. An individual heterozygous for one mutation may also be heterozygous for another mutation (compound heterozygotes); therefore, it is possible that, if the reference group with genotype WT/WT may contain three other types of people, namely those with H63D/H63D, C282Y/H63D and H63D/WT. As a consequence, Ellervik et al. used incorrect study-specific odds ratios because they did not take into account this difference in the genotyping frequencies, and therefore, overestimated the pooled estimates for the C282Y/C282Y ($OR_{\text{Ellervik}}: 10.42$, 99% CI: 2.05, 52.99; $OR_{\text{corrected}}: 5.87$, 99% CI: 1.47, 23.48) and for the mixed heterozygotes genotypes ($OR_{\text{Ellervik}}: 3.30$, 99% CI: 1.40, 7.70; $OR_{\text{corrected}}: 1.47$, 99% CI: 1.01, 2.12). In contrast we found similar estimates for the H63D homozygotes ($OR_{\text{Ellervik}}: 1.30$, 99% CI: 0.49, 3.43; $OR_{\text{corrected}}: 1.25$, 99% CI: 0.50, 3.10) (Figure S5). When we applied those corrections and replicated the Ellervik et al. meta-analysis focusing on their search period, we did not find any association between *HFE* and NAFLD (data not shown).

The lack of power, different ethnic variation, information and selection biases and the presence of confounders have been frequently quoted to explain the inconsistency across *HFE*-related studies [20, 58, 64]. A meta-analysis is a possible solution to overcoming the issue of power. In our study of 1,727 NAFLD Caucasian cases and 4,275 controls, we had 88% power to detect an OR of 1.20 assuming a prevalence of any *HFE* mutation in the reference population of 36.9% (estimated from our findings) and a two-sided alpha error of 0.05 [65]. Therefore, it is likely that even if *HFE* variants had an effect on the presence of NAFLD, the effect size would be relatively small. We also showed that information/selection biases did not substantially influence our results. Our results showed a trend to increased odds in non Caucasian populations, however, this finding deserves more studies since the overall estimate is driven by largest of the three studies [36, 46, 53].

The current analysis has several limitations. First of all, we weren't able to address whether the *HFE* genetic variants acted as a disease-modifying gene in NASH (e.g. compare

genotypes in simple steatosis only to those in NASH) or the impact of *HFE* genetic variants on iron biomarkers in NAFLD patients. The published data were insufficient to address such question; and remains unsolved [58, 66]. Our definition of ethnicity was somehow arbitrary but, we believe, was consistent with the reported literature. It also reflects the unusual reporting of ethnicity across studies, a key variable in genetic studies. Third, the extrapolation of these results in the general population is somehow difficult because the majority of the case-control studies came from the hospital setting (where cases were defined using histology). Finally, when we examined statistical heterogeneity across different clinically relevant characteristics as previously described, our inference of no association between the presence of any *HFE* mutation and NAFLD remained unchanged. We acknowledged that we did not have enough characteristics of each study to correct for the presence of statistical heterogeneity. Only cooperation between researchers and individual data meta-analysis could address this question using meta-regression or genome wide association studies.

In contrast, our meta-analysis has several strengths. Not only we systemically reviewed the literature but also we obtained primary data from authors if needed. Compared to Ellervik, our current initiative focus and expands our knowledge on the association between the presence of *HFE* genetic variants and NAFLD.

In conclusion, we have found no association between the *HFE* genotype and NAFLD in Caucasians and non-Caucasians. We believe the *HFE* may have none or, at maximum, a marginal role in the development of NAFLD. Future studies should focus on the role of *HFE* in the progression of NAFLD and its association with iron biomarkers (peripheral and hepatic).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations

NALFD	nonalcoholic fatty liver disease
C282Y	cysteine-to-tyrosine substitution at the amino acid 282
H63D	histidine-to-aspartate change at the amino acid 63
HFE	hemochromatosis gene
OMIM	online Mendelian inheritance in man
MeSH	Medical subheadings
EMTREE	Embase thesaurus
NHW	non-Hispanic whites
NHANES III	Third National Health and Nutrition Examination Survey
WT	wild type

STREGA	STrengthening the Reporting of Genetic Associations
MOOSE	Meta-Analysis of Observational Studies in Epidemiology
OR	odds ratio
NASH	nonalcoholic steatohepatitis
CI	confidence interval
N/A	not applicable
N/R	not reported
U/S	ultrasounds

References

1. Lazo M, Clark JM. The epidemiology of nonalcoholic fatty liver disease: a global perspective. *Semin Liver Dis.* 2008 Nov; 28(4):339–350. [PubMed: 18956290]
2. American Gastroenterological Association. American Gastroenterological Association medical position statement: nonalcoholic fatty liver disease. *Gastroenterology.* 2002 Nov; 123(5):1702–1704. [PubMed: 12404244]
3. Brunt EM. Nonalcoholic steatohepatitis: definition and pathology. *Semin Liver Dis.* 2001; 21(1):3–16. [PubMed: 11296695]
4. Day CP, James OF. Steatohepatitis: a tale of two “hits”? *Gastroenterology.* 1998 Apr; 114(4):842–845. [PubMed: 9547102]
5. Day CP. Genes or environment to determine alcoholic liver disease and non-alcoholic fatty liver disease. *Liver Int.* 2006 Nov; 26(9):1021–1028. [PubMed: 17032401]
6. Brunet S, Thibault L, Delvin E, Yotov W, Bendayan M, Levy E. Dietary iron overload and induced lipid peroxidation are associated with impaired plasma lipid transport and hepatic sterol metabolism in rats. *Hepatology.* 1999 Jun; 29(6):1809–1817. [PubMed: 10347124]
7. Silva M, Silva ME, de PH, Carneiro CM, Pedrosa ML. Iron overload alters glucose homeostasis, causes liver steatosis, and increases serum triacylglycerols in rats. *NUTR RES.* 2008 Jun; 28(6):391–398. [PubMed: 19083437]
8. Fernandez-Real JM, Lopez-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. *Diabetes.* 2002 Aug; 51(8):2348–2354. [PubMed: 12145144]
9. Guyader D, Deugnier Y. Steatosis, iron and HFE genes. *J Hepatol.* 2007 Dec 31.
10. Tsuchiya H, Akechi Y, Ikeda R, Nishio R, Sakabe T, Terabayashi K, et al. Suppressive Effects of Retinoids on Iron-Induced Oxidative Stress in the Liver. *Gastroenterology.* 2009; 136(1):341–350. [PubMed: 18952085]
11. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet.* 1996 Aug; 13(4):399–408. [PubMed: 8696333]
12. Steinberg KK, Cogswell ME, Chang JC, Caudill SP, McQuillan GM, Bowman BA, et al. Prevalence of C282Y and H63D mutations in the hemochromatosis (HFE) gene in the United States. *JAMA.* 2001 May 2; 285(17):2216–2222. [PubMed: 11325323]
13. Adams PC, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, et al. Hemochromatosis and iron-overload screening in a racially diverse population. *N Engl J Med.* 2005 Apr 28; 352(17):1769–1778. [PubMed: 15858186]
14. Cogswell ME, McDonnell SM, Khoury MJ, Franks AL, Burke W, Brittenham G. Iron overload, public health, and genetics: Evaluating the evidence for hemochromatosis screening. *Ann Intern Med.* 1998; 129(11):971–979. [PubMed: 9867750]
15. Samarasekera J, Winsor W, Lush R, Duggan P, Xie Y, Borgaonkar M. Individuals homozygous for the H63D mutation have significantly elevated iron indexes. *Dig Dis Sci.* 2006; 51(4):803–807. [PubMed: 16615007]

16. Walsh A, Dixon JL, Ramm GA, Hewett DG, Lincoln DJ, Anderson GJ, et al. The clinical relevance of compound heterozygosity for the C282Y and H63D substitutions in hemochromatosis. *Clin Gastroenterol Hepatol*. 2006 Nov; 4(11):1403–1410. [PubMed: 16979952]
17. Ellervik C, Birgens H, Tybjaerg-Hansen A, Nordestgaard BG. Hemochromatosis genotypes and risk of 31 disease endpoints: meta-analyses including 66,000 cases and 226,000 controls. *Hepatology*. 2007 Oct; 46(4):1071–1080. [PubMed: 17828789]
18. Hernaez, R.; Yeung, E.; Clark, JM.; Kowdley, KV.; Brancati, FL.; Kao, W. [Accessed 01-15-2009] HFE gene and nonalcoholic fatty liver disease: a systematic review and meta-analysis. 2007 Nov 28. http://www.cdc.gov/genomics/hugenet/reviews_prep.htm
19. Equitani F, Fernandez-Real JM, Menichella G, Koch M, Calvani M, Nobili V, et al. Bloodletting ameliorates insulin sensitivity and secretion in parallel to reducing liver iron in carriers of HFE gene mutations. *Diabetes Care*. 2008 Jan; 31(1):3–8. [PubMed: 17959863]
20. Chitturi S, Weltman M, Farrell GC, McDonald D, Kench J, Liddle C, et al. HFE mutations, hepatic iron, and fibrosis: ethnic-specific association of NASH with C282Y but not with fibrotic severity. *Hepatology*. 2002 Jul; 36(1):142–149. [PubMed: 12085358]
21. George DK, Goldwurm S, MacDonald GA, Cowley LL, Walker NI, Ward PJ, et al. Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. *Gastroenterology*. 1998 Feb; 114(2):311–318. [PubMed: 9453491]
22. Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, von EE, et al. Strengthening the Reporting of Genetic Association studies (STREGA): an extension of the STROBE Statement. *Ann Intern Med*. 2009 Feb 3; 150(3):206–215. [PubMed: 19189911]
23. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA*. 2000; 283(15):2008–2012. [PubMed: 10789670]
24. Feder JN, Tsuchihashi Z, Irrinki A, Lee VK, Mapa FA, Morikang E, et al. The hemochromatosis founder mutation in HLA-H disrupts beta2-microglobulin interaction and cell surface expression. *J Biol Chem*. 1997 May 30; 272(22):14025–14028. [PubMed: 9162021]
25. Tomatsu S, Orii KO, Fleming RE, Holden CC, Waheed A, Britton RS, et al. Contribution of the H63D mutation in HFE to murine hereditary hemochromatosis. *Proc Natl Acad Sci U S A*. 2003 Dec 23; 100(26):15788–15793. [PubMed: 14673107]
26. Distant S, Robson KJ, Graham-Campbell J, rnaiz-Villena A, Brissot P, Worwood M. The origin and spread of the HFE-C282Y haemochromatosis mutation. *Hum Genet*. 2004 Sep; 115(4):269–279. [PubMed: 15290237]
27. Milman N, Pedersen P. Evidence that the Cys282Tyr mutation of the HFE gene originated from a population in Southern Scandinavia and spread with the Vikings. *Clin Genet*. 2003 Jul; 64(1):36–47. [PubMed: 12791037]
28. Lazo M, Clark JM. The epidemiology of nonalcoholic fatty liver disease: a global perspective. *Semin Liver Dis*. 2008 Nov; 28(4):339–350. [PubMed: 18956290]
29. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ*. 2003 Sep 6; 327(7414):557–560. [PubMed: 12958120]
30. Higgins JP. Commentary: Heterogeneity in meta-analysis should be expected and appropriately quantified. *Int J Epidemiol*. 2008 Oct; 37(5):1158–1160. [PubMed: 18832388]
31. Higgins, JP.; Green, S. *Cochrane Handbook for Systematic Reviews of Interventions*. 2008. Version 5.0.1 ed
32. Song F, Sheldon TA, Sutton AJ, Abrams KR, Jones DR. Methods for exploring heterogeneity in meta-analysis. *Eval Health Prof*. 2001 Jun; 24(2):126–151. [PubMed: 11523383]
33. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L. Comparison of two methods to detect publication bias in meta-analysis. *JAMA*. 2006 Feb 8; 295(6):676–680. [PubMed: 16467236]
34. Hernaez R, Yeung E, Clark JM, Kowdley KV, Brancati FL, Kao WHL. HFE and nonalcoholic fatty liver disease: A systematic review and meta-analysis. *Hepatology*. 2009; 50:781A. [PubMed: 19575453]

35. Aigner E, Theurl I, Haufe H, Seifert M, Hohla F, Scharinger L, et al. Copper availability contributes to iron perturbations in human nonalcoholic fatty liver disease. *Gastroenterology*. 2008 Aug; 135(2):680–688. [PubMed: 18505688]
36. Lee SH, Jeong SH, Lee D, Lee JH, Hwang SH, Cho YA, et al. An epidemiologic study on the incidence and significance of HFE mutations in a Korean cohort with nonalcoholic fatty liver disease. *J Clin Gastroenterol*. 2010 Aug; 44(7):e154–e161. [PubMed: 20216079]
37. Bonkovsky HL, Jawaideh 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. *J Hepatol*. 1999 Sep; 31(3):421–429. [PubMed: 10488699]
38. Bonkovsky HL, Zivny J, Tortorelli K, Liu Q, Lambrecht L. Non-alcoholic steatohepatitis with iron: part of insulin resistance-associated hepatic iron overload? (Reply). *J Hepatol*. 2000 Dec; 33(6):1024–1026. [PubMed: 11131442]
39. Bugianesi E, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, et al. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology*. 2004 Jan; 39(1):179–187. [PubMed: 14752836]
40. Floreani A, Rosa RE, Basso D, Navaglia F, Zaninotto M, Petridis I, et al. An open population screening study for HFE gene major mutations proves the low prevalence of C282Y mutation in Central Italy. *Aliment Pharmacol Ther*. 2007 Aug 15; 26(4):577–586. [PubMed: 17661761]
41. Lin TJ, Lin CL, Wang CS, Liu SO, Liao LY. Prevalence of HFE mutations and relation to serum iron status in patients with chronic hepatitis C and patients with nonalcoholic fatty liver disease in Taiwan. *World J Gastroenterol*. 2005 Jul 7; 11(25):3905–3908. [PubMed: 15991291]
42. Mendler MH, Turlin B, Moirand R, Jouanolle AM, Sapey T, Guyader D, et al. Insulin resistance-associated hepatic iron overload. *Gastroenterology*. 1999 Nov; 117(5):1155–1163. [PubMed: 10535879]
43. Neri S, Pulvirenti D, Santo S, Luca I, Aikaterini T, Mauceri B, et al. The HFE gene heterozygosis H63D: a cofactor for liver damage in patients with steatohepatitis? Epidemiological and clinical considerations. *Intern Med J*. 2007 Oct 3.
44. Perez-Aguilar F, Benlloch S, Berenguer M. Study of patients referred for elevated ferritin levels and/or transferrin saturation: significance of non-alcoholic fatty liver disease. *Gastroenterol Hepatol*. 2004 Nov; 27(9):508–514. [PubMed: 15544735]
45. Pucelikova T, Cieslarova B, Putova I, Hrubá I, Stritesky J, Horak J. Prevalence of HFE gene C282Y and H63D mutations and elevated iron biochemistries in patients with non-alcoholic fatty liver and alcoholic liver disease. *Ceska Slov Gastroenterol Hepatol*. 2004; 58(3):89–93.
46. Simsek H, Balaban YH, Sumer H, Yilmaz E, Tatar G. HFE mutations analysis of Turkish patients with nonalcoholic steatohepatitis. *Dig Dis Sci*. 2006 Oct; 51(10):1723–1724. [PubMed: 16964543]
47. Valenti L, Dongiovanni P, Piperno A, Fracanzani AL, Maggioni M, Rametta R, et al. Alpha 1-antitrypsin mutations in NAFLD: high prevalence and association with altered iron metabolism but not with liver damage. *Hepatology*. 2006 Oct; 44(4):857–864. [PubMed: 17006922]
48. Venat L, Loustaud-Ratti V, Liozon E, Soria P, Nadalon S, Gissot V, et al. Dysmetabolic hepatosiderosis, characteristics in 51 patients. *Presse Med*. 2003 Mar 8; 32(9):400–405. [PubMed: 12712916]
49. Zamin I Jr, Mattos AA, Mattos AZ, Migon E, Bica C, Alexandre CO. Prevalence of the hemochromatosis gene mutation in patients with nonalcoholic steatohepatitis and correlation with degree of liver fibrosis. *Arq Gastroenterol*. 2006 Jul; 43(3):224–228. [PubMed: 17160239]
50. Bacon BR, Olynyk JK, Brunt EM, Britton RS, Wolff RK. HFE genotype in patients with hemochromatosis and other liver diseases. *Ann Intern Med*. 1999 Jun 15; 130(12):953–962. [PubMed: 10383365]
51. Brunt EM, Olynyk JK, Britton RS, Janney CG, Di Bisceglie AM, Bacon BR. Histological evaluation of iron in liver biopsies: relationship to HFE mutations. *Am J Gastroenterol*. 2000 Jul; 95(7):1788–1793. [PubMed: 10925986]
52. Deguti MM, Sipahi AM, Gayotto LC, Palacios SA, Bittencourt PL, Goldberg AC, et al. Lack of evidence for the pathogenic role of iron and HFE gene mutations in Brazilian patients with nonalcoholic steatohepatitis. *Braz J Med Biol Res*. 2003 Jun; 36(6):739–745. [PubMed: 12792703]

53. Duseja A, Das A, Das R, Dhiman RK, Chawla Y, Bhansali A, et al. The clinicopathological profile of Indian patients with nonalcoholic fatty liver disease (NAFLD) is different from that in the West. *Dig Dis Sci*. 2007 Sep; 52(9):2368–2374. [PubMed: 17420951]
54. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology*. 2006 Oct; 44(4):865–873. [PubMed: 17006923]
55. Laroussi N, Mosnier JF, Morel Y, Deugnier Y, Dumas O, Audigier JC. Non alcoholic steatohepatitis: a multifactorial, frequent, paucysymptomatic liver disease with a fibrotic outcome. *Gastroenterol Clin Biol*. 2002 May; 26(5):475–479. [PubMed: 12122357]
56. Loria P, Lonardo A, Carulli N. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology*. 2004 Jun; 39(6):1748. [PubMed: 15185321]
57. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes*. 2001 Aug; 50(8):1844–1850. [PubMed: 11473047]
58. Nelson JE, Bhattacharya R, Lindor KD, Chalasani N, Raaka S, Heathcote EJ, et al. HFE C282Y mutations are associated with advanced hepatic fibrosis in Caucasians with nonalcoholic steatohepatitis. *Hepatology*. 2007 Sep; 46(3):723–729. [PubMed: 17680648]
59. Perez-Carerras M, Castellano G, Perez E, Morales P, Yela C, Rodriguez S, et al. Iron status and HFE gene mutations in nonalcoholic steatohepatitis (NASH). *J Hepatol*. 2002; 36(S1):150.
60. Sikorska K, Bielawski KP, Stalke P, Lakomy EA, Michalska Z, Witczak-Malinowska K, et al. HFE gene mutations in Polish patients with disturbances of iron metabolism: an initial assessment. *Int J Mol Med*. 2005 Dec; 16(6):1151–1156. [PubMed: 16273299]
61. Yamauchi N, Itoh Y, Tanaka Y, Mizokami M, Minami M, Morita A, et al. Clinical characteristics and prevalence of GB virus C, SEN virus, and HFE gene mutation in Japanese patients with nonalcoholic steatohepatitis. *J Gastroenterol*. 2004 Jul; 39(7):654–660. [PubMed: 15293136]
62. Yoneda M, Nozaki Y, Endo H, Mawatari H, Iida H, Fujita K, et al. Serum Ferritin Is a Clinical Biomarker in Japanese Patients with Nonalcoholic Steatohepatitis (NASH) Independent of HFE Gene Mutation. *Dig Dis Sci*. 2009 Mar 7.
63. Raszeja-Wyszomirska J, Kurzawski G, Lawniczak M, Miezynska-Kurtycz J, Lubinski J. Nonalcoholic fatty liver disease and HFE gene mutations: a Polish study. *World J Gastroenterol*. 2010 May 28; 16(20):2531–2536. [PubMed: 20503453]
64. Younossi ZM, Gramlich T, Bacon BR, Matteoni CA, Boparai N, O'Neill R, et al. Hepatic iron and nonalcoholic fatty liver disease. *Hepatology*. 1999 Oct; 30(4):847–850. [PubMed: 10498632]
65. Dupont, WD.; Plummer, WD. PS power and sample size program available for free on the Internet. 18. 1997. p. 274
66. Valenti L, Fracanzani AL, Bugianesi E, Dongiovanni P, Galmozzi E, Vanni E, et al. HFE Genotype, Parenchymal Iron Accumulation, and Liver Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology*. 2010 Mar; 138(3):905–912. [PubMed: 19931264]

Figure.
Odds Ratio and 95% Confidence Intervals for the Presence of *HFE* Mutation in NAFLD And Controls, Chronologically Ordered.

Table 1

Case-Control Studies of *HFE* Gene and NAFLD (Chronologically Ordered)

Author, year	Country	Ethnicity	Inclusion	Outcome (% NASH)	Confirmation	Pathological criteria	NAFLD (N)	Controls (N)	Source controls
George, 1998[21]	Australia	Caucasian	N/R	NASH (100)	Pathology	Scheuer	51	2,465	Brisbane newborns and healthy laboratory workers
Mendler, 1999[42]	France	Caucasian	Hepatic iron overload	NAFLD (52)	Pathology	Independent	86	75	Random healthy adults
Bonkovsky, 2000[37, 38]	U.S.A.	Caucasian	Enzymes or imaging	NASH (100)	Pathology	Goodman	57	348	Historic controls
Chitturi, 2002[20]	Australia	Caucasian	Enzymes	NASH (100)	Pathology	Brunt	42	127	Blood donors
Venat, 2003[48]	France	Caucasian	Hyperferritinemia or bright liver	NAFLD	U/S or pathology	N/R	12	9	Normal ultrasound
Bugianesi, 2004[39]	Italy	Caucasian	Enzymes	NAFLD (33)	U/S or pathology	Brunt	210	204	Blood donors
Perez-Agular, 2004[44]	Spain	Caucasian	Hyperferritinemia	NAFLD	U/S	N/A	22	21	Normal ultrasound
Pucelikova, 2004[45]	Czech Republic	Caucasian	N/R	NALFD	U/S or pathology	N/A	41	257	Randomly selected newborns
Lin, 2005[41]	Taiwan	Asian	Enzymes and U/S	NAFLD	U/S	N/A	33	125	Healthy check-up controls
Simsek, 2006[46]	Turkey	Asian	N/R	NAFLD (100)	Pathology	N/R	30	2677	Newborns
Valenti, 2009[47]	Italy	Caucasian	Enzymes, imaging	NASH (100)	Pathology	Brunt	594	184	Blood donors
Zamin, 2006[49]	Brazil	Caucasian	Enzymes	NASH (100)	Pathology	Brunt	29	20	No peptic disease and normal enzyme levels
Floreani, 2007[40]	Italy	Caucasian	Ultrasound	NAFLD	U/S	N/A	171	205	No liver imaging
Neri, 2007[43]	Italy	Caucasian	Enzymes and U/S	NASH (100)	Pathology	Brunt	272	430	Blood donors (no steatosis)
Aigner, 2008[35]	Austria	Caucasian	N/R	NAFLD (16)	Pathology	Kleiner	61	20	Liver biopsies unexplained etiology
Lee, 2010[36]	Korea	Asian	Health screening	NAFLD	U/S	N/A	125	221	No ultrasound lesion

Abbreviations: N/A: not applicable, N/R: not reported; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; U/S: ultrasound

Table 2Case Only Studies of *HFE* Gene and NAFLD (Chronologically Ordered)

Author, year	Country	Inclusion	Outcome (definition)	Confirmation	Pathological criteria	NAFLD (N)
Bacon, 1999[50]	U.S.A.	Liver disease	NASH	Pathology	N/R	9
Brunt, 2000[51]	U.S.A.	Stainable iron in liver biopsy	NASH	Pathology	N/R	15
Marchesini, 2001[57]	Italy	Enzymes	NAFLD	Pathology	Diehl	30
Laroussi, 2002[55]	France	N/R	NASH	Pathology	Lee	25
Perez-Carreras, 2002[59]	Spain	N/R	NASH	Pathology	N/R	60
Deguti, 2003[52]	Brazil	Enzymes	NASH	Pathology	Independent	31
Loria, 2004[56]	Italy	Ultrasound	NAFLD	Pathology	N/R	122
Yamauchi, 2004[61]	Japan	N/R	NAFLD	Pathology	Brunt	27
Sikorska, 2005[60]	Poland	Chronic liver disease with iron overload	NAFLD	Pathology	Independent	6
Eksstedt, 2006[54]	Sweden	Enzymes	NAFLD	Pathology	Brunt	129
Duseja, 2007[53]	India	Enzymes, imaging	NAFLD	U/S or pathology	Matteoni and Brunt	30
Nelson, 2007[58]	U.S.A. and Canada	N/R	NASH	Pathology	Brunt	126
Yoneda, 2009[62]	Japan	N/R	NAFLD	Pathology	Brunt	78
Raszeja-Wyszomirska, 2010[63]	Poland	N/R	NAFLD	Pathology	N/R	62

Abbreviations: N/A: not applicable, N/R: not reported; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; U/S: ultrasound