Distribution of Transferrin Saturation in an Australian Population: Relevance to the Early Diagnosis of Hemochromatosis

CHRISTINE E. McLAREN,* GEOFFREY J. McLACHLAN,† JUNE W. HALLIDAY,§ SONJA I. WEBB,§ BARBARA A. LEGGETT,§ ELIZABETH C. JAZWINSKA,§ DARRELL H. G. CRAWFORD,§ VICTOR R. GORDEUK,‖ GORDON D. McLAREN,¶ and LAWRIE W. POWELL§

*Department of Mathematics, Moorhead State University, Moorhead, Minnesota; †Department of Mathematics, University of Queensland, Brisbane, Australia; §Joint Liver Program, The Queensland Institute of Medical Research, Brisbane, Australia; ‖Division of Hematology and Oncology, The George Washington University Medical Center, Washington, D.C.; and ¶Department of Veterans Affairs Medical Center and University of North Dakota School of Medicine and Health Sciences, Fargo, North Dakota

Background & Aims: An elevated transferrin saturation is the earliest phenotypic abnormality in hereditary hemochromatosis. Determination of transferrin saturation remains the most useful noninvasive screening test for affected individuals, but there is debate as to the appropriate screening level. The aims of this study were to estimate the mean transferrin saturation in hemochromatosis heterozygotes and normal individuals and to evaluate potential transferrin saturation screening levels. *Methods:* Statistical mixture modeling was applied to data from a survey of asymptomatic Australians to estimate the mean transferrin saturation in hemochromatosis heterozygotes and normal individuals. To evaluate potential transferrin saturation screening levels, modeling results were compared with data from identified hemochromatosis heterozygotes and homozygotes. *Results:* After removal of hemochromatosis homozygotes, two populations of transferrin saturation were identified in asymptomatic Australians ($P < 0.01$). In men, 88.2% of the truncated sample had a lower mean transferrin saturation of 24.1%, whereas 11.8% had an increased mean transferrin saturation of 37.3%. Similar results were found in women. A transferrin saturation threshold of 45% identified 98% of homozygotes without misidentifying any normal individuals. *Conclusions:* The results confirm that hemochromatosis heterozygotes form a distinct transferrin saturation subpopulation and support the use of transferrin saturation as an inexpensive screening test for hemochromatosis. In practice, a fasting transferrin saturation of ≥45% identifies virtually all affected homozygous subjects without necessitating further investigation of unaffected normal individuals.

Hereditary hemochromatosis (HC) is the most common autosomal recessively inherited disorder in white populations.[1–9] Past surveys have estimated the gene frequency of HC by identifying homozygotes in the population.[4–6,10] Using this approach, Leggett et al.[7] surveyed a non–blood donor, predominantly white (of European descent) Australian population and estimated the prevalence of iron overload attributable to HC to be 0.36%, with a corresponding gene frequency of 0.0592. An alternative approach to estimate the gene frequency, based on statistical mixture modeling of transferrin saturation, was recently described by McLaren et al.[9] This technique can be used to separate an observed distribution into the component subpopulations. Application of mixture modeling to transferrin saturation distributions in the second National Health and Nutrition Examination Survey yielded an estimated hemochromatosis gene frequency of 0.070–0.081 in the U.S. population.

Studies of family members have suggested that mean transferrin saturation values for heterozygotes are higher than among unaffected subjects but lower than among homozygotes,[1–3,8,11,12] although a recent study asserted that mean transferrin saturation values are the same for unaffected individuals and heterozygotes.[13] In the present investigation, we applied statistical mixture modeling to the data from the Australian study to examine the distribution of transferrin saturation in this population and to estimate the mean transferrin saturation in heterozygotes for HC. These findings were then compared with the mean transferrin saturation in HC heterozygotes identified from pedigree analysis including HLA studies.[8]

A sustained transferrin saturation level of >62% has been proposed to screen for homozygotes.[14,15] We used statistical mixture modeling to identify the most appropriate level of transferrin saturation to maximize identifi-

Abbreviation used in this paper: HC, hereditary hemochromatosis.

0016-5085/98/$3.00

cation of HC homozygotes while minimizing misidentification of HC heterozygotes and unaffected normal individuals.

Materials and Methods

Data From a Population of Asymptomatic Individuals

Subjects were asymptomatic Australians previously studied to determine the prevalence of homozygous HC. The population was composed of employees of two large Australian companies, a banking corporation and an insurance corporation; 96.7% were white and the remainder were Australian Aboriginal or Asian. Venous blood was obtained by venipuncture from nonfasting volunteers between 9 and 11 AM, and serum was stored at −20°C until analysis. Additional demographic details and the methods used for measurements of serum biochemical tests of iron status have been described previously.[7] Subjects were screened initially by determination of serum iron concentration and total iron binding capacity, from which transferrin saturation was calculated, and serum ferritin concentration. Subjects who had an initial transferrin saturation of >45% were recalled. Transferrin saturation and serum ferritin concentration measurements were repeated, and, if transferrin saturation had decreased below 45%, no further follow-up was undertaken. If it remained above 45%, subjects were interviewed and examined. Those with a consistently elevated serum ferritin concentration (men >200 mg/L; women >150 mg/L) underwent percutaneous needle biopsy of the liver and assessment of hepatic iron concentration.[16] In subjects with elevated transferrin saturation but normal serum ferritin concentration, the transferrin saturation test was repeated in the fasting state; if the results were still abnormal, the tests were repeated every 6 months and liver biopsy was performed if serum ferritin concentration showed a progressive increase. Subjects with elevated serum ferritin concentration but transferrin saturation of <45% were not recalled because it was not considered ethical to perform liver biopsy on asymptomatic individuals with no family history of HC unless the diagnosis was strongly suggested by elevation of both measurements. Seven subjects were identified as having HC.[7]

Selection Criteria for Data From a Population of Asymptomatic Individuals

In the current study, we examined data from subjects in an asymptomatic Australian population who were at least 20 years of age. This subsample consisted of 891 white men and 761 women, aged 20–73 years. We selected transferrin saturations computed from blood samples in which the mean red blood cell volume was between 80 and 100 fL and serum ferritin level was >10 μg/L. Additional selection criteria included transferrin saturation values from men with a hemoglobin level of ≥14 g/dL and hematocrit level of ≥42% and from women with a hemoglobin level of ≥12 g/dL and hematocrit level of ≥36%. We then excluded subjects having persistently high transferrin saturation values who also drank 80–100 g of alcohol per day because elevated transferrin saturation may be associated with hepatocellular damage caused by alcohol.[17] We excluded subjects with abnormally low hemoglobin, hematocrit, ferritin, or mean corpuscular volume values because anemias of various causes may be associated with abnormally high transferrin saturations[18–20] or low transferrin saturations.[21–23] Because abnormally high mean corpuscular volume can be associated with megaloblastic conditions and drug effects,[24,25] all of which can lead to altered transferrin saturations,[21,24–26] such subjects were also excluded.

Because an objective of our study was to determine if the distribution of transferrin saturation values from asymptomatic individuals contained a subpopulation of values consistent with that of HC heterozygotes, we excluded the 4 men and 3 women with proven HC to ensure that the final data set of 796 men and 669 women (Table 1) would include homozygous normal and heterozygous subjects.

Estimation of Prevalence of Hemochromatosis Heterozygotes

We used the Hardy–Weinberg equilibrium equation, $p^2 + 2pq + q^2 = 1$, to calculate the prevalence of heterozygotes in the population of asymptomatic individuals based on a gene frequency of $q = 0.0592$.[7] Given that $p = 1 - q = 0.9408$, the proportion of heterozygotes in the total population is then calculated as $2pq = 0.11$.[27] The scaled proportion of heterozygotes in the population of asymptomatic individuals, excluding HC homozygotes, would be 11.8%.

Distribution of Transferrin Saturation Values From Asymptomatic Australians

We examined separately the distributions of transferrin saturation values among the remaining 796 white men and 669 women from the sample data using techniques developed for analysis of mixtures of distributions.[28] Because transferrin saturation is normally distributed in normal subjects,[9] the physiological models used were a single normal distribution and a mixture of two normal distributions. Given that the scaled prevalence of heterozygotes in asymptomatic Australians was 11.8%, mixing proportions for the mixture model were specified for homozygous normal and heterozygous subjects to be 0.882 and 0.118, respectively. The expectation-maximiza-

Table 1. Exclusions From a Sample of Banking and Insurance Corporation Employees 20 Years of Age or Older

	Men	Women
Total sample	891	761
Excluded for		
Proven hemochromatosis	4	3
Low ferritin	7	40
Low hemoglobin	67	36
Low hematocrit	6	8
Abnormal MCV	8	5
Persistently elevated transferrin saturation and moderately heavy alcohol intake	3	
Final analytic sample	796	669

MCV, mean corpuscular volume.

tion algorithm, an iterative technique, was applied to the distributions of transferrin saturation values to estimate the model parameters (means and standard deviations).[28–30]

The statistical test used to determine the best fitting model was based on the likelihood ratio statistic. For each observed distribution, the maximized log-likelihood function for a mixture of normal distributions was evaluated ($\log L_1$) and compared with the maximized log-likelihood function for a single normal distribution ($\log L_0$). Significance of the likelihood ratio statistic, $-2\log(L_0/L_1)$, was assessed using a resampling technique.[31,32] Resampling is a method that can be used to compute P values based on the observed data instead of referring to theoretical probabilities. A P value of <0.05 indicated a better fit to a mixture of two normal distributions than to a single normal distribution. The χ^2 statistic was then used to test goodness of fit of each observed distribution to the best fitting model.

Data From Hemochromatosis Homozygotes and Heterozygotes and Evaluation of Potential Transferrin Saturation Screening Values

For comparison with data from the population of asymptomatic Australians, we also examined the variability in transferrin saturation values from an additional population of 485 subjects known to be homozygous or heterozygous for HC.[8,16,33,34] We examined successive transferrin saturation thresholds. We calculated the proportion of homozygotes above a given threshold and the corresponding proportion of heterozygotes below that threshold. These results were compared with the population mixture modeling of data from the asymptomatic individuals to evaluate potential transferrin saturation screening levels. For a given threshold, we calculated the predicted proportion of unaffected individuals below the threshold and the predicted proportion of heterozygotes above the threshold.

Results

Distribution of Transferrin Saturation Values in a Population of Asymptomatic Individuals

After removal of values from identified HC homozygotes, and assuming a scaled heterozygote proportion of 11.8%, two distinct transferrin saturation populations could be identified in data from 796 men (likelihood ratio statistic, 53.81; $P < 0.01$; goodness-of-fit $\chi^2 = 14.5$, $df = 16$, $P = 0.56$) and 669 women (likelihood ratio statistic, 89.3; $P < 0.01$; goodness-of-fit $\chi^2 = 21.7$, $df = 20$, $P = 0.36$), as shown in Figure 1 and Table 2. For men, a major subpopulation corresponding to 88.2% of the truncated sample had a mean transferrin saturation (± 1 SD) of 24.1% $\pm$ 6.0% and may predominantly include normal subjects. A second subpopulation comprising 11.8% of the truncated sample had an increased mean transferrin saturation of 37.3% $\pm$ 7.7% and may represent predominantly individuals who are heterozygous for the HC gene. Similarly, analysis of data from women showed respective subpopulations of the truncated sample with mean transferrin saturations (± 1 SD) of 22.5% $\pm$ 6.4% and 37.6% $\pm$ 10.4%.

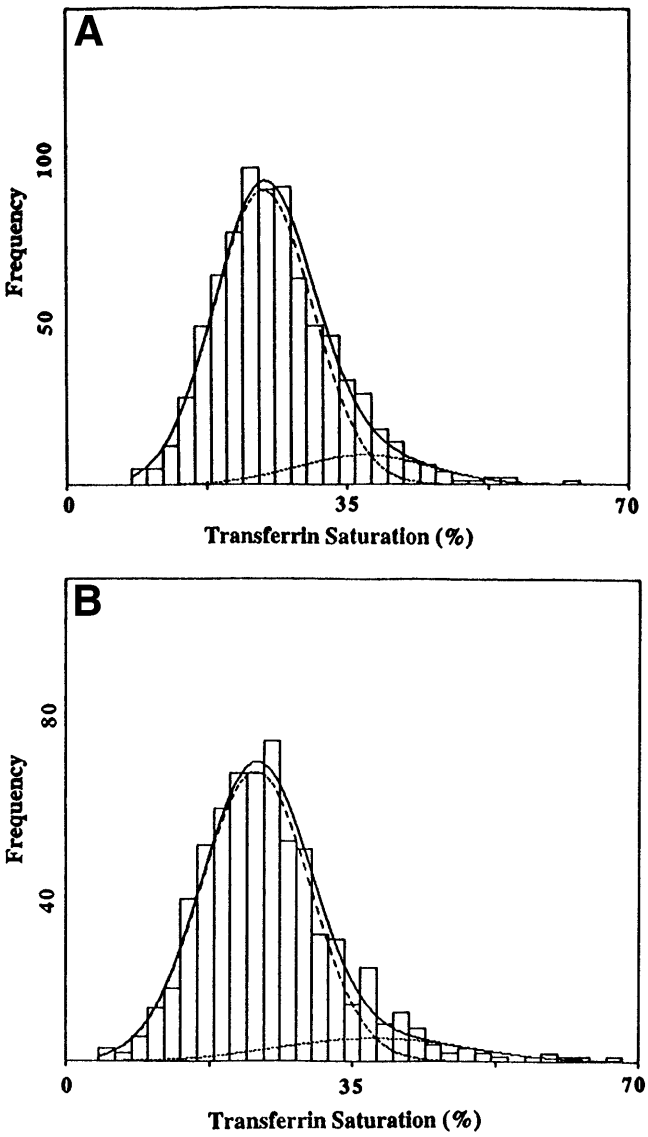

Figure 1. Distribution of transferrin saturation values among asymptomatic Australians: (*A*) 796 men and (*B*) 669 women. The *dashed lines* represent the fitted curves for individual subpopulations. The overall fitted mixture distribution is shown with a *solid line.*

Table 2. Transferrin Saturation

	Asymptomatic individuals		Individuals identified by pedigree analysis	
Sex	Postulated unaffected	Postulated heterozygotes	Known heterozygotes	Known homozygotes
Male	24.1 ± 6.0	37.3 ± 7.7	37.1 ± 17.0	82.7 ± 14.4
Female	22.5 ± 6.4	37.6 ± 10.4	32.5 ± 15.3	75.3 ± 19.3

NOTE. Results are expressed as mean ± SD percentage.

Variability of Transferrin Saturation Values in a Population of Hemochromatosis Homozygotes and Heterozygotes

The transferrin saturation distributions of 142 homozygous HC probands and 343 heterozygotes were examined. In homozygous probands, the minimum and maximum transferrin saturation values for 107 men were 48% and 100% with a mean (±1 SD) of 82.7% ± 14.4% and a median of 85%. For 35 women, the minimum and maximum transferrin saturation values were 39% and 100%, with a mean of 75.3% ± 19.3% and a median of 80%. In HC homozygotes, the variability in transferrin saturation values could not be explained by differences in age or any other characteristic. There was no significant correlation between age and transferrin saturation for men (Pearson's $r = 0.075$, $P = 0.45$) or women (Pearson's $r = -0.253$, $P = 0.14$). Results for heterozygotes showed that, for 157 men, the mean transferrin saturation value was 37.1% ± 17.0% with a minimum of 13%, a maximum of 95%, and a median of 35%. For 186 women, the mean transferrin saturation value was 32.5% ± 15.3% with a minimum of 7%, a maximum of 95%, and a median of 30%.

Evaluation of Potential Transferrin Saturation Screening Values

Separate analyses of data from the population of HC homozygote probands and heterozygotes were performed for men and women. A screening level of 45% classified 100% of male homozygotes correctly; 45 of 157 heterozygotes (28.7%) also had a transferrin saturation above 45%. In contrast, a screening level of 60% correctly classified 96 of 107 (89.7%) of homozygotes, but 11 of 157 heterozygotes (7%) also had a transferrin saturation above 60%.

For women, a screening level of 45% classified 31 of 35 female homozygotes (91.4%) correctly, whereas 31 of 186 heterozygotes (16.7%) also had transferrin saturations above 45%. A screening level of 60% correctly classified only 26 of 35 homozygotes (74.3%), whereas 14 of 186 heterozygotes (7.5%) also had transferrin saturations above 60%.

These results were compared with population mixture modeling of data from the asymptomatic individuals to find transferrin saturation levels that would maximize identification of HC homozygotes while minimizing misidentification of heterozygotes and unaffected individuals. The scaled proportions in two subpopulations suggested that the distribution of 1465 asymptomatic individuals was composed of 1292 postulated unaffected individuals and 173 postulated heterozygotes for HC (Figure 1). The area under each fitted curve, above and below selected transferrin saturation levels, was computed for each subpopulation. The results in Table 3 show that if a screening value of 40% were to be used, a predicted 5 of 1292 of the postulated unaffected individuals (0.4%) would undergo further testing unnecessarily. By using a transferrin saturation of 45%, none of postulated unaffected individuals would undergo further testing unnecessarily and 33 of 173 of the heterozygote (19.1%) would be identified. These predictions can be compared with the observed sensitivity, 98% for identification of true homozygote probands, and 76 of 343 (22.2%), for identification of true heterozygotes. As the screening value is raised, the positive predictive accuracy increases, but the sensitivity of the test for identification of homozygotes decreases. Using the known HC heterozygotes and homozygotes, a screening value of 45% gave a positive predictive accuracy for homozygote detection of 65% (139 of 215), whereas use of the screening value of 60% gave a positive predictive value of 122 of 147 (83%) but a decreased sensitivity of 122 of 142 (85.9%).

Discussion

There is now incontrovertible evidence that early diagnosis and therapy of HC prevents virtually all manifestations of the disease and results in normal life expectancy.[35,36] In contrast, unrecognized and untreated disease leads to cirrhosis, hepatocellular carcinoma, and other lethal complications. Heterozygotes for HC with coexisting hematologic disorders such as idiopathic refractory sideroblastic anemia, hereditary spherocytosis, pyruvate kinase deficiency,[37–40] or sporadic porphyria cutanea tarda[41] may develop iron overload sufficient to cause overt organ damage.[35,36,42] In addition, when a putative heterozygote is identified, this provides the opportunity to conduct studies of family members to identify individu-

Table 3. Transferrin Saturation Levels in Postulated Unaffected and Heterozygous Subjects Compared With Known Heterozygous and Homozygous Individuals

Transferrin saturation (%)	Postulated unaffected[a] n (% of 1292)	Postulated heterozygotes[a] n (% of 173)	Known heterozygotes[b] n (% of 343)	Known homozygotes n (% of 142)
≥40	5 (0.4)	59 (34.1)	102 (29.7)	141 (99.3)
≥45	0 (0.0)	33 (19.1)	76 (22.2)	139 (97.9)
≥50	0 (0.0)	13 (7.5)	53 (15.5)	135 (95.1)
≥55	0 (0.0)	5 (2.9)	34 (9.9)	131 (92.3)
≥60	0 (0.0)	1 (0.6)	25 (7.3)	122 (85.9)
≥62	0 (0.0)	1 (0.6)	20 (5.8)	120 (84.5)

[a]Derived from the statistical modeling of transferrin saturation data from asymptomatic Australians.

[b]These individuals were identified by pedigree studies and HLA typing of known probands with hemochromatosis.

als who are homozygous for the disease. Thus, early identification of heterozygotes and homozygotes for hemochromatosis is an important clinical challenge.

Evaluation of family members has suggested that the mean transferrin saturation in heterozygotes is higher than among unaffected subjects but lower than in homozygotes.[1–3,11] This result has been questioned.[13] In this study, we applied statistical mixture modeling to population data and compared mean transferrin saturation values in subpopulations with those from identified HC heterozygotes and homozygotes from pedigree studies. Modeling results support the conclusion that the means for transferrin saturations in male and female heterozygotes differ from those in unaffected subjects (Figure 1). In analysis of data from male subjects, two populations could be detected; one, comprising 88.2% with a mean ± SD transferrin saturation of 24.1% ± 6.0%, may predominantly include individuals who are unaffected, and the second subpopulation with 11.8% of the values and an increased mean transferrin saturation of 37.3% ± 7.7% may be composed predominantly of individuals who are heterozygous for the HC gene. Similar results were found in women; 88.2% of the values had a mean of 22.5% ± 6.4% and may include individuals who are unaffected by the gene for HC, whereas the second subpopulation with 11.8% of the values had an increased mean transferrin saturation of 37.6% ± 10.4% and may represent predominantly individuals who are heterozygous for the HC gene. For comparison, in the Australian population of 343 known heterozygotes, the mean transferrin saturation was 37.1% ± 17.0% for males and 32.5% ± 15.3% for females (Table 2). For men and women, the estimated variance of transferrin saturation values was smaller among postulated heterozygotes than among known heterozygotes. A likely explanation of this is that the heterozygotes in the pedigree analysis had a high frequency of the ancestral haplotype,[12] which is known to be associated with more severe clinical expression.

During the study of asymptomatic individuals with transferrin saturation initially above 45% but with subsequent values below 45%, no further follow-up was undertaken. Thus, some HC homozygotes could have been missed and the prevalence of homozygotes underestimated. Before data analysis, we excluded subjects with persistently high transferrin saturation values who also drank 80–100 g of alcohol per day. Some of these exclusions may have included HC heterozygotes or homozygotes.

Determination of transferrin saturation is the most useful method of screening for affected homozygous individuals,[14,43] but there is considerable debate as to the screening values that should be used. In our study, the computed mean values for known heterozygotes and homozygotes were within published limits: 37.1% for male and 32.5% for female HC heterozygotes and 82.7% for male and 75.3% for female homozygous probands. Examination of mean values does not reveal the extent of variability of values for transferrin saturation. For example, among homozygous male probands, 12 of 107 of the transferrin saturation values (11.2%) were below 62%, including values from 48% to 61%. Among homozygous female probands, 10 of 35 of the values (29%) were below 62%, ranging from 39% to 61%. Heterogeneity of disease expression in HC has been described[44] due in part to the inheritance of an ancestral haplotype,[12] dietary intake,[43] and other unknown factors.[45]

The upper limit of normal for transferrin saturation frequently quoted is 50%,[46] with a level of >62% strongly suggestive of the homozygous state.[14,15] Our data suggest that an upper limit of 45% is the lowest screening value that could be used for identifying individuals at risk for HC without unnecessary testing of unaffected normal individuals. Based on mixture modeling analysis, we found that if a transferrin saturation of 45% were used for screening, none of the postulated unaffected individuals would undergo further testing unnecessarily and approximately 19% of the postulated heterozygotes would be identified. For comparison, using this transferrin saturation screening level, 98% of true HC homozygotes with expression of the disease and 22% of true HC heterozygotes were identified. Our analyses confirm that, as the screening value is raised, the sensitivity of the test for identification of homozygotes decreases. For example, a threshold of 60% would identify only 86% of the known homozygotes (Table 3).

In our study, for 76 identified heterozygotes with a transferrin saturation of >45%, 14 of 45 men (31.1%) and 26 of 31 women (83.9%) had serum ferritin levels within the normal range (men <200, women <150 μg/L). Thus, we found that heterozygotes can have increased transferrin saturation but normal ferritin levels. It should be noted that asymptomatic individuals in this study with a transferrin saturation >45% but a normal serum ferritin level did not undergo liver biopsy at the time of initial screening. This is consistent with a previous study showing that most individuals with elevated transferrin saturation but normal serum ferritin level would not undergo such further evaluation, because serum ferritin levels usually were not elevated in heterozygotes.[47] For practical purposes, it is important to detect those heterozygotes with increased iron stores who would then be evaluated further. Therefore, cost-effectiveness

would be increased by the addition of serum ferritin evaluation, and this could be done conveniently at the time of a second, confirmatory transferrin saturation determination.

Analysis of transferrin saturation data from asymptomatic Australian individuals, truncated to remove values from identified HC homozygotes, shows that two subpopulations of individuals could be detected, consistent with one subpopulation of predominately unaffected individuals and one of HC heterozygotes. These data also support the use of transferrin saturation as a screening test for HC to identify possible affected individuals for further testing. The results indicate that the statistical methods employed in this study can be used to predict the outcome of using particular transferrin saturation thresholds for screening in different populations and to determine appropriate reference values. In practice, a fasting transferrin saturation of $\geq$45% identifies 98% of affected homozygous subjects with the disease.

A recent publication by Feder et al.[48] on a strong candidate gene for HC highlights the need for a simple, inexpensive screening test for phenotypic expression of the disease. The mutant gene has been identified in most patients with hemochromatosis.[48–51] A DNA-based diagnostic test that might arise from identification of the HC gene is likely to be expensive, especially if the technique involves use of the polymerase chain reaction. Thus, a test for the candidate gene would need to be applied selectively to putatively affected individuals identified by a simple reliable test of disease expression. Transferrin saturation fills that role.

References

1. Kravitz K, Skolnick M, Cannings C, Carmelli D, Baty B, Amos B, Johnson A, Mendall N, Edwards C, Cartwright G. Genetic linkage between hereditary hemochromatosis and HLA. Am J Hum Genet 1979;31:601–619.
2. Edwards CQ, Dadone MM, Skolnick MH, Kushner JP. Hereditary hemochromatosis. Clin Haematol 1982;11:411–435.
3. Borwein ST, Ghent CN, Flanagan PR, Chamberlain MJ, Valberg LS. Genetic and phenotypic expression of hemochromatosis in Canadians. Clin Invest Med 1983;6:171–179.
4. Tanner AI, Desai S, Lu W, Wright R. Screening for haemochromatosis in the UK. Preliminary results (abstr). Gut 1985;26:1139.
5. Karlsson M, Ikkala E, Reunanen A, Takkunen H, Vuori E, Mäkinen J. Prevalence of haemochromatosis in Finland. Acta Med Scand 1988;224:385–390.
6. Hallberg L, Bjorn-Rasmussen E, Jungner I. Prevalence of hereditary haemochromatosis in two Swedish urban areas. J Intern Med 1989;225:249–255.
7. Leggett BA, Halliday JW, Brown NN, Bryant S, Powell W. Prevalence of haemochromatosis amongst asymptomatic Australians. Br J Haematol 1990;74:525–530.
8. Summers KM, Halliday JW, Powell LW. Identification of homozygous hemochromatosis subjects by measurement of hepatic iron index. Hepatology 1990;12:20–25.
9. McLaren CE, Gordeuk VR, Looker AC, Hasselblad V, Edwards CQ, Griffen LM, Kushner JP, Brittenham GM. Prevalence of heterozygotes for hemochromatosis in the white population of the United States. Blood 1995;86:2021–2027.
10. Elliot R, Tait A, Lin BPC, Smith CI, Dent OF. Prevalence of haemochromatosis in a random sample of asymptomatic men. Aust NZ J Med 1986;16:491–495.
11. Porto G, Vicente C, Fraga J, Martins de Silva B, De Sousa M, Hemochromatosis Clinical and Research Group. Importance of establishing appropriate local reference values for the screening of hemochromatosis: a study of three different control populations and 136 hemochromatosis family members. J Lab Clin Med 1992;19:295–305.
12. Crawford DHG, Powell LW, Leggett BA, Francis JS, Fletcher LM, Webb SI, Halliday JW, Jazwinska EC. The ancestral haplotype in Australian hemochromatosis patients is associated with severe clinical expression. Am J Hum Genet 1995;57:362–367.
13. Borecki IB, Rao DC, Yaouanq J, Lalouel JM. Percent transferrin saturation in segregating hemochromatosis. Am J Med Genet 1990;36:301–305.
14. Dadone MM, Kushner JP, Edwards CQ, Bishop DT, Skolnick MH. Hereditary hemochromatosis: analysis of laboratory expression of the disease by genotype in 18 pedigrees. Am J Clin Pathol 1982;78:196–207.
15. Edwards CQ, Kushner JP. Screening for hemochromatosis. N Engl J Med 1993;328:1616–1620.
16. Bassett ML, Halliday JW, Powell LW. Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron concentration associated with fibrosis. Hepatology 1986;6:24–29.
17. Powell LW, Fletcher LM, Halliday JW. Distinction between haemochromatosis and alcoholic siderosis. In: Hall P, ed. Alcoholic liver disease. London: Edward Arnold, 1995:205.
18. Cartwright GE, Huguley CM, Ashenbrucker H, Fay J, Wintrobe MM. Studies on free erythrocyte protoporphyrin, plasma iron and plasma copper in normal and anemic subjects. Blood 1948;3:501–525.
19. Finch CA, Deubelbeiss K, Cook JD, Eschbach JW, Harker LA, Funk DD, Marsaglia G, Hillman RS, Slichter S, Adamson JW, Ganzoni A, Giblett ER. Ferrokinetics in man. Medicine 1970;49:17–53.
20. Hershko C, Graham G, Bates GW, Rachmilewitz EA. Non-specific serum iron in thalassaemia: an abnormal serum iron fraction of potential toxicity. Br J Haematol 1978;40:255–263.
21. Cartwright GE, Wintrobe MM. Chemical, clinical, and immunological studies on the products of human plasma fractionation. XXXIX. The anemia of infection. Studies on the iron-binding capacity of serum. J Clin Invest 1949;28:86–98.
22. Bainton DF, Finch CA. The diagnosis of iron deficiency anemia. Am J Med 1964;37:62–70.
23. Cook JD, Lipschitz DA, Miles LEM, Finch CA. Serum ferritin as a measure of iron stores in normal subjects. Am J Clin Nutr 1974;27:681–687.
24. Jandl JH. Blood. Boston: Little, Brown, 1987:153–155.
25. Oski FA. Anemia due to other nutritional deficiencies. In: Beutler E, Lichtman MA, Coller BS, Kipps TJ, eds. Williams hematology. 5th ed. New York: McGraw Hill, 1995:511–515.
26. Gordeuk VR, Brittenham GM, McLaren GD, Spagnuolo PJ. Hyperferremia in immunosuppressed patients with acute nonlymphocytic leukemia and the risk of infection. J Lab Clin Med 1986;108:466–472.
27. Cavalli-Sforza LL, Bodmer WF. The genetics of human populations. San Francisco: Freeman, 1971:234–238.
28. McLaren CE, Wagstaff M, Brittenham GM, Jacobs A. Detection of two component mixtures of lognormal distributions in grouped doubly-truncated data. Biometrics 1991;47:607–622.
29. Dempster AP, Laird NM, Rubin DB. Maximum likelihood from incomplete data via the EM algorithm. J R Stat Soc B 1977;39:1–38.

30. Hasselblad V. Manual for the continuous DISFIT software. Version 1.1. Durham, NC: Duke University, 1994.
31. McLachlan GJ, Basford KE. Mixture Models: inference and applications to clustering. New York: Dekker, 1988:25–26.
32. McLachlan GJ, McLaren CE, Matthews D. An algorithm for the likelihood ratio test of one versus two components in a normal mixture model fitted to grouped and truncated data. Commun Stat: Simul Comput 1995;24:965–985.
33. Jazwinska EC, Lee SC, Webb SI, Halliday JW, Powell LW. Localization of the hemochromatosis gene close to D6S105. Am J Human Genet 1993;53:347–352.
34. Jazwinska EC, Pyper WR, Burt MJ, Francis JS, Goldwurm S, Webb SI, Lee SC, Halliday JW, Powell LW. Haplotype analysis in Australian hemochromatosis patients: evidence for a predominant ancestral haplotype exclusively associated with hemochromatosis. Am J Human Genet 1995;56:428–433.
35. Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, Strohmeyer G. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. N Engl J Med 1985;313:1256–1262.
36. Niederau C, Fischer R, Purschel A, Stremmel W, Haussinger D, Strohmeyer G. Long-term survival in patients with hereditary hemochromatosis. Gastroenterology 1996;110:1304–1307.
37. Cartwright GE, Edwards CQ, Skolnick MH, Amos DB. Association of HLA-linked hemochromatosis with idiopathic refractory sideroblastic anemia. J Clin Invest 1980;65:989–992.
38. Fargion S, Cappellini MD, Piperno A, Panajotopoulos N, Ronchi G, Fiorelli G. Association of hereditary spherocytosis and idiopathic hemochromatosis: a synergistic effect in determining iron overload. Am J Clin Pathol 1986;86:645–649.
39. Mohler DN, Wheby MS. Hemochromatosis heterozygotes may have significant iron overload when they also have hereditary spherocytosis. Am J Med Sci 1986;292:320–324.
40. Zanella A, Berzuini A, Colombo MB, Guffanti A, Lecchi L, Cappellini MD, Barosi G. Iron status in red cell pyruvate kinase deficiency: study of Italian cases. Br J Haematol 1993;83:485–490.
41. Kushner JP, Edwards CQ, Dadone MM, Skolnick MH. Heterozygosity for HLA-linked hemochromatosis as a likely cause of the hepatic siderosis associated with sporadic porphyria cutanea tarda. Gastroenterology 1985;88:1232–1238.
42. Bradbear RA, Bain C, Siskind V, Schofield FD, Webb S, Axelsen EM, Halliday JW, Bassett ML, Powell LW. Cohort study of internal malignancy in genetic hemochromatosis and other chronic nonalcoholic liver diseases. J N Can Inst 1985;75:81–84.
43. Powell LW, Jazwinska E, Halliday JW. Primary iron overload. In: Brock JH, Halliday JW, Pippard MJ, Powell LW, eds. Iron metabolism in health and disease. Philadelphia: Saunders, 1994:247–254.
44. Muir WA, McLaren GD, Braun W, Askari A. Evidence for heterogeneity in hereditary hemochromatosis: evaluation of 174 persons in 9 families. Am J Med 1984;76:806–814.
45. Gordeuk VR, McLaren GD, Samowitz W. Etiologies, consequences and treatment of iron overload. Crit Rev Clin Lab Sci 1994;31:89–133.
46. McLaren GD. Iron-overload disorders. In: Mazza JJ, ed. Manual of clinical hematology. Boston: Little, Brown, 1995:128.
47. Bulaj ZJ, Griffen LM, Jorde LB, Edwards CQ, Kushner JP. Clinical and biochemical abnormalities in people heterozygous for hemochromatosis. N Engl J Med 1996;335:1799–1805.
48. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R, 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, Prass CE, Quintana L, Starnes SM, Schatzman RC, Brunke KJ, Drayna DT, Risch NJ, Bacon BR, Wolff RK. A novel MHC class 1–like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996;13:399–408.
49. Beutler E, Gelbart T, West C, Lee P, Adams M, Blackstone R, Pockros P, Kosty M, Venditti CP, Phatak PD, Seese NK, Chorney KA, Elshof AE, Gerhard GS, Chorney M. Mutational analysis in hereditary hemochromatosis. Blood Cells Mol Dis 1996;22:187–194.
50. Jazwinska EC, Cullen LM, Busfield F, Pyper WR, Webb SI, Powell LW, Morris CP, Walsh TP. Haemochromatosis and *HLA-H*. Nat Genet 1996;14:249–251.
51. Jouanolle AM, Gandon G, Jezequel P, Blayau M, Campion ML, Yaouanq J, Mosser J, Fergelot P, Chauvel B, Bouric P, Carn G, Andrieux N, Gicquel I, Le Gall J-Y, David V. Haemochromatosis and *HLA-H*. Nat Genet 1996;14:251–252.

Received January 17, 1997. Accepted November 18, 1997.

Address requests for reprints to: Christine McLaren, Ph.D., Division of Epidemiology, Department of Medicine, University of California, Irvine, 224 Irvine Hall, Irvine, California 92697-7550. Fax: (714) 824-4773.

Supported in part by a Senior International Fellowship 1 F06 TW02117 awarded by the Fogarty International Center of the National Institutes of Health and an Ethyl Raybould Fellowship awarded by the Department of Mathematics at the University of Queensland (to C.E.M.). Additional support was provided by the National Health and Medical Research Council of Australia and the Department of Veterans Affairs.

The authors thank Drs. S. Bryant and N. Brown for assistance in the Australian population study.