

Impact of HLA-H Mutations on Iron Stores in Healthy Elderly Men and Women

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ABSTRACT: The DNA of 287 healthy white elderly volunteers in the New Mexico Aging Process Study, between 63 and 91 years of age, was examined for mutations of the HLA-H gene at nt 845 and nt 187. None were found to be homozygous for the 845A mutation and there were no gender differences in the percentage of the various mutations. The frequency of the 845A mutation was 0.061 resulting in a carrier frequency of 12.2%. The frequency of the 187G mutation was 0.136 resulting in a carrier frequency of 19.9% for a single mutation; 2.4% were compound heterozygous, 845A/187G and 2.4% were homozygous for the 187G mutation. After excluding 5 men and 4 women with microcytic or macrocytic anemia, mean percent transferrin saturation (PSAT) and iron stores, as estimated from serum ferritin concentrations, were calculated for each mutation. Estimated iron stores were normally distributed (range ~50 to 1,550 mg) with men (n=111) having significantly higher mean estimated iron stores than women (n=167), 826±318 and 753±287 mg, respectively. More men, 15 of 28, (54%) with estimated iron stores in the upper quartile, ≥1,050 mg, had a HLA-H mutation compared to 25 of 83 (30%) who had a mutation and whose estimated iron stores were <1,050 mg, p<0.05. Seven were heterozygous for the 845A mutation with mean estimated iron stores of 1,300±127 mg, 7 were heterozygous for the 187G mutation with mean estimated iron stores of 1,233±165 mg and 1 was compound heterozygous with estimated iron stores of 1,439 mg. Similar differences were not noted in women. Even though the potential role of the 187G mutation in the phenotypic expression of HH is less certain than the 845A mutation, the increase in PSAT seen in men with the 187G mutation and the equal distribution of 845A and 187G mutations seen with iron stores ≥1,050 mg lends support for the involvement of the 187G mutation, or a linked mutation, in iron metabolism. We concluded that men having either a single chromosomal 845A and/or 187G mutation results in higher PSAT's and estimated iron stores than if no HLA-H mutation were present.

Keywords: HLA-H, ageing, iron stores, PSAT, serum transferrin saturation, serum ferritin, hemochromatosis

INTRODUCTION

Hereditary hemochromatosis (HH) is an autosomal recessive disorder leading to excessive accumulation of iron in various organs of the

body over time. In 1996, Feder et al. identified a candidate gene for HH, termed HLA-H, related to the major histocompatibility complex class I family of genes (1). Feder et al. found that HLA-H contained two missense alterations, one

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was a G to A transition at nucleotide 845 that results in a Cys→Tyr substitution at amino acid 282 (C282Y); the second was a C to G transition at nucleotide 187 that results in a His→Asp acid substitution at amino acid 63 (H63D). In their study of 178 patients clinically diagnosed with HH, Feder et al. found that 148 were homozygous and 9 were heterozygous for the 845A mutation. Twenty one of the patients were homozygous for the usual allele (845G); however, 9 of these subjects were found to be heterozygous and 1 was found to be homozygous for the 187G mutation. Thus, 83% of their HH patients were HLA-H 845A homozygous which led Feder et al. (1) to conclude that HLA-H was a very strong candidate for the HH gene. Feder et al. also found that 8 out of 9 patients with one chromosomal 845A mutation were compound heterozygotes (845A/187G). Only 10 of 155 control subjects (6.4%) carried one chromosomal 845A mutation and 51 (33%) carried one chromosomal 187G mutation while only one control (~1%) was found to be homozygous for the 187G variant.

Beutler et al. (2) examined 147 patients clinically diagnosed with HH, along with 193 control subjects, for mutations at nucleotides 845 and 187. Eighty-two percent (n=121) of the HH patients were found to be homozygous and 6.8% (n=10) were found to be heterozygous for the 845A mutation. Eight of the 10 heterozygotes for 845A were found to be compound heterozygotes (845A/187G). None of the control subjects were homozygous for the 845A; however, 29 (15%) were heterozygous for 845A and 47 (24.3%) were heterozygous for the 187G mutation. Two of the control subjects (1%) were found to be compound heterozygotes (845A/187G). These findings were very similar to those reported by Feder et al. in that approximately 83% of the clinically diagnosed HH patients were found to be homozygous for the 845A mutation, and that most of the subjects diagnosed with HH who were heterozygous for 845A were, in fact, compound heterozygotes (845A/187G).

More recently, Barton et al. (3) examined 74 subjects with HH and found 44 (59.5%) were

845A homozygotes. However, when they segregated the probands who had two or more of the following alternate criteria: hepatic iron concentration >4500 µg/g dry weight; hepatic iron index >2.0; grade 3 or 4+ hepatocellular iron; and >4 g of iron removed by phlebotomy, 36 of the 44 probands (81.9%) met this criteria. They also examined 142 normal control subjects and found the frequencies of the various genotypes were similar to those of Beutler et al. (2).

Studies conducted in European countries, namely the United Kingdom (4), France (5) and Italy (6) have also confirmed the very strong association between HH and HLA-H even though differences in the gene frequencies for the 845A and 187G between these European populations varied considerably among their control populations. These previous reports (1-6) were primarily designed to examine the gene frequencies of the 845A and 187G mutations in patients clinically diagnosed with HH. None examined the possible gender differences of the HLA-H mutations and whether normal control individuals who are heterozygous for either mutation, compound heterozygotes or homozygous for the 187G mutation exhibited increases in percent transferrin saturation and iron stores.

The primary purpose of the present study was to examine the frequency of the 845 and 187 mutations in a well defined group of elderly men and women enrolled in the New Mexico Aging Process Study (NMAPS). The NMAPS is a longitudinal study of nutrition and aging. We were also interested in determining whether iron stores, as determined by serum ferritin levels, along with other measures of iron status, e.g., percent transferrin saturation and iron intake, explain some of the variances noted in these measurements when comparing those carrying only the normal alleles with those found to be carrying either the 845A or 187G mutations, as well as those found to be compound heterozygotes, 845A/187G, and those homozygous for the 187 G mutation.

MATERIALS AND METHODS

Subjects

Entrance into the NMAPS is limited to persons aged 60 years or older with no known serious medical conditions; e.g., persons with diagnosed cancer, aortic stenosis, diabetes or transient ischemic attack were excluded. Persons with osteoarthritis or cataracts were included if they were not taking prescription medications for these or other ailments. Once accepted into the study, subsequently diagnosed illnesses and medication use did not result in the elimination of subjects from the study. The subjects' ages ranged from 60 to 98 years in 1995 with a median age of 77 years for both males and females. Entrance was not limited to any ethnic group but all volunteers were white; 3% were of Hispanic origin. More than 40% had college degrees. For a more complete description of the population, the reader is referred to previous publications (7-9). The volunteers were seen as outpatients in the Clinical Nutrition Program at the University of New Mexico Health Sciences Center. After an overnight fast, -50 mL of blood was obtained from each person between 0800 and 0930 hours for various hematological, molecular biology and biochemical measurements. For this study we used laboratory measurements of iron status (CBC, serum iron, TIBC, PSAT, and ferritin) collected yearly from 1993 to 1996. Subjects for this study were 171 women and 116 men who were HLA-H genotyped in 1997.

This study was approved by the Human Research Review Committee of the University of New Mexico School of Medicine. Informed consent was obtained from each participant.

Laboratory Measurements

Blood chemistries. Complete blood count measurements were conducted using a Coulter "S" instrument (Coulter Electronics, Hialeah FL). Serum iron and total iron binding capacity (TIBC) were measured by an automated

colorimetric method using sulfonated bathophenanthroline as the chromogen (10). Transferrin saturation (PSAT) was calculated by expressing the serum iron concentration as a percent of the TIBC concentration. Serum ferritin determinations were performed using an immunoassay (LPIA-Ferritin Assay, Seradyn, Inc., Indianapolis, IN). The measurements of iron status (serum iron, TIBC, and ferritin) were monitored for long-term reproducibility using a lyophilized human serum control (Lypocheck, Bio-Rad Laboratories, Richmond, CA). The average coefficients of variation (CVs) for serum iron measurements over a period of 1 year were 3.6 and 2.5 percent for iron concentrations of 95 $\mu\text{g/dL}$ (17 $\mu\text{mol/L}$) and 191 $\mu\text{g/dL}$ (34.2 $\mu\text{mol/L}$), respectively. The average CVs for TIBC measurements over a period of 1 year were 7.3 and 6.5 percent for TIBC levels of 269 $\mu\text{g/dL}$ (48.2 $\mu\text{mol/L}$) and 365 $\mu\text{g/dL}$ (63.4 $\mu\text{mol/L}$), respectively. The inter-assay CVs for ferritin with low (25.5 $\mu\text{g/L}$), medium (160 $\mu\text{g/L}$) and high (391 $\mu\text{g/L}$) concentrations in 10 separate assays were 1.9, 2.8 and 3.6 percent, respectively.

Mutation analyses. The procedure of Feder et al. was used for restriction enzyme fragment length polymorphism genotyping of the 845A and 187G alleles associated with hemochromatosis (1). To isolate genomic DNA, whole blood (5ml) is drawn into EDTA tubes, centrifuged, and the buffy coat isolated. Cells are suspended in lysis buffer (100 mM NaCl, 10 mM Tris.HCl, pH 8.0, 25 mM EDTA, 0.05% SDS, 0.1 mg/ml proteinase K) and incubated at 37C overnight. The supernatants are phenol:chloroform extracted and the DNA is then ethanol precipitated and resuspended in Tris EDTA buffer. PCR assays are performed in a final volume of 50 μl using genomic DNA (1 μg), 0.2 μM of each primer, PCR amplification buffer (1X), 1.5 mM MgCl_2 , 0.2 mM dNTP, and 1.25U of Taq polymerase. PCR assay conditions consist of an initial denaturation of 2 minutes (94C), 30 cycles of denaturation (94C, 1 minute), primer annealing for the 845 mutation (62C, 1 minute), primer

annealing for the 187 mutation (60C, 1 min), extension (72C, 1 minute), followed by a final extension for 4 minutes (72C). The PCR primers used for amplification are: 845 position - Forward: 5'-TGGCAAGGGTAAACAGATC C-3', Reverse: 5'-CTCAGGCACTC CTCTCAA CC-3'; 187 position - Forward: 5'-ACATGGTT AAGGCCTGTTGC-3', Reverse: 5'-GCCACA TCTGGCTTGAAATT-3'. The 845 PCR amplification yields a 390bp product. Ras I digested PCR products are resolved on a 2.5% Nusieve agarose gel (FMC, Rockland, ME) and alleles assigned as: (Wild type) normal allele:240bp --150bp; heterozygous: 240bp --150bp -- 120bp -- 30bp; homozygous: 240bp --- 120bp -- 30bp. The 187 PCR amplification yields a 200bp product. Mbo I digested PCR products are resolved on a 2.5% Nusieve agarose gel and alleles assigned as: (Wild type) normal allele: 140bp -- 60bp; heterozygous: 200bp -- 140bp -- 60bp; homozygous:200bp.

Dietary intake measurements. A research nutritionist gave volunteers instruction for keeping an accurate 3-day food record. Each volunteer was asked to measure all food eaten for three consecutive weekdays and to record the intake on standard coding forms. Brand names, methods of food preparation, and recipes for any mixed dish eaten during the period were also recorded. Commercial plastic food models were used as instructional aids to assist the volunteers in judging portion sizes. An electronic diet scale and an instruction booklet designed for this study were also provided. This booklet stressed the need for accuracy, completeness, and recording food items prepared from a recipe.

At the end of the three-day recording period, a research nutritionist visited the volunteers' homes to review the diet records. At this time, each record was subjectively evaluated for completeness and accuracy, and the participants were asked to provide additional information about any unclear food item. If vitamin or mineral supplements were used by the subject, the brand name, contents, and amounts of each nutrient

were recorded for inclusion in determining total intakes. All food records were coded by food item and amount and then analyzed for nutrient composition. Data were analyzed using the Food Intake Analysis System (FIAS), version 2.0 (University of Texas at Houston). These records were then used to estimate the subjects iron intakes.

Estimates of body iron. We estimated iron stores by the method of Cook et al. (11). Different levels of iron status require separate calculations to estimate body iron as described in detail in our previous publication (12). For subjects with serum ferritin concentrations above 12 µg/L, the method reduces to the following equation:

$$\text{iron stores (mg)} = 400 \times (\ln \text{SF} - \ln 12)$$

where 400 is the proportionality constant, ln is the natural logarithm and SF is serum ferritin in µg/L. All of the subjects had ferritin levels above 12 µg/L except for two women.

Statistical methods. Statistical analyses were conducted using SAS (version 6.12, SAS Institute, Cary, NC). We used Pierson chi-square statistics to test the percentage of various genotypes for gender differences. The statistical significance of associations between genotypes and PSAT and iron stores was assessed using general linear models using SAS procedure GLM for men and women separately. We examined for known confounding variables that could influence PSAT and ferritin levels, a surrogate for determining iron stores, e.g., alcohol intake, inflammatory processes, as determined by erythrocyte sedimentation rates and total iron intake both in subjects with the usual genotype and those with a HLA-H mutation. Logistic regression was used to estimate the odds of having high iron stores (>75%) for those with any mutation versus those with the usual genotype.

RESULTS

Table 1 shows the percentage of various genotypes for the entire population (n=287). As expected, there were no significant gender differences in the percentage of the various genotypes in this population since the frequencies of autosomal genes should be the same in men and women unless there is a selected mortality rate associated with either the 845A or 187G mutations. The frequency of the 845A allele was

0.061 (35/574) resulting in a carrier frequency of 12.2% (35/287). The frequency of the 187G allele was 0.136 (78/574) resulting in a carrier frequency of 19.9% (57/287) for a single 187G mutation; 2.4% for compound (845A/187G) heterozygotes, and 2.4% for homozygous 187G/187G. Our data are consistent with that noted in other American control subjects as summarized in Table 2 even though they were from different regions of the United States.

Table 1. Number and percent of each HLA-H genotype for women (n=171) and men (n=116) enrolled in the NMAPS.

Genotype	Women		Men		Total	
	n	%	n	%	n	%
845G/187C	113	66.1	75	64.7	188	65.5
845G/187G	35	20.5	22	19.0	57	19.9
845A/187C	16	9.4	12	10.3	28	9.8
845A/187G	3	1.7	4	3.4	7	2.4
187G/187G	4	2.3	3	2.6	7	2.4
Total	171	100	116	100	287	100

Table 2. Frequencies of the various HLA-A genotypes of control populations in the NMAPS and those of Beutler et al. (2) and Barton et al. (3).

	NMAPS	Beutler et al. (2)	Barton et al. (3)	Total
Genotype	n (%)	n (%)	n (%)	n (%)
845A/845A	0 (0)	0 (0)	1 (0.7)	1 (0.2)
845G/187C	188 (65.5)	112 (58.0)	89 (62.7)	389 (62.5)
845G/187G	57 (19.9)	45 (23.3)	28 (19.7)	130 (20.9)
845A/187C	28 (9.8)	27 (14.0)	15 (10.6)	70 (11.2)
845A/187G	7 (2.4)	2 (1.0)	5 (3.5)	14 (2.3)
187G/187G	7 (2.4)	7 (3.6)	4 (2.8)	18 (2.9)
Total	287 (100)	193 (100)	142 (100)	622 (100)

Table 3. Mean (SD) PSAT and estimated iron stores (IS) in women and men in the NMAPS by genotype.

Genotype	Women			Men		
	n	PSAT	IS (mg)	n	PSAT	IS (mg)
845G/187C	110	27.2±7.1	743±284	71	29.3±7.9	776±300
845G/187G	34	33.7±7.9	751±259	21	37.0±9.2	910±280
845A/187C	16	30.1±9.6	698±325	12	36.2±7.2	997±412
845A/187G	3	46.0±7.9	1,193±225	4	38.5±11.0	839±453
187G/187G	4	33.2±5.3	954±204	3	36.3±4.0	740±225

Based on the combined frequency for the 845A and 187G alleles noted in Table 2, the Hardy Weinberg equilibrium would predict that in the general population 1 out of every 208 (0.48%) individuals should be homozygous for the 845A mutation, 1 out of every 48 (2.1%) individuals should be homozygous for the 187G mutation and 1 out of every 50 (2.0%) individuals should be compound heterozygotes (845A/187G). The finding that fewer than predicted individuals were found to be homozygous for the 845A mutation reflects the fact that the NMAPS population has a median age of ~77 years and any 845A homozygous individuals most likely would have demonstrated clinical symptoms associated with HH and would not have met the entrance criteria for the NMAPS.

Figure 1 shows the frequency distribution of iron stores (mg) for the men (n=111) and women (n=167) after excluding the 9 individuals who had either macrocytic (MCV>100) or microcytic (MCV<85) anemia. Eight of these individuals were homozygous for the 842G allele and one was heterozygous for the 187G allele (187C/187G). Iron stores were normally distributed and men had significantly higher (p=0.048) mean iron stores than women, 826 ± 318(SD) and 753±287 mg, respectively. The median value for men was 814 mg and for women it was 722 mg.

Table 3 shows the mean (SD) percent transferrin saturation (PSAT) and iron stores by

genotype for the women and men. Because of the limited number of subjects in the various cells noted in Table 3, we were unable to conduct a valid statistical comparison between those within a specific genotype and those with the usual genotype for differences in PSAT and iron stores by gender. However, we did compare women (n=110) and men (n=71) with the usual genotype with the remaining women (n=57) and men (n=40) expressing an 845 or 187 mutation, including compound heterozygotes and homozygotes for the 187G mutation. We found no individuals who were homozygous for the 845 mutation.

Women (n=57) with HLA-H mutations had significantly higher (p<0.001) PSAT's (33.0±9.0%) compared to women (n=110) with the usual genotype (27.2±7.1%). Similar to the women, men (n=40) with a mutation had significantly higher (p<0.001) PSAT's than men (n=71) with the usual genotype, 36.9±8.3 and 29.3±7.9%, respectively. Over 50% of those with PSAT's in the upper tertile, (>32% and >36% for women and men, respectively) had either a 845A or 187G mutation. Women with any mutation had iron stores that were not significantly different from those with the usual genotype, i.e., 754±325 and 743±284 mg, respectively. However, men with any mutation had significantly increased iron stores compared to those men with the usual genotype, 916±334 and 776±300 mg, respectively, (p=0.025). Adjusting iron stores for age,

height and weight did not change the level of significance in iron stores between the two groups of men, i.e., usual genotype versus any mutation.

To test whether iron stores were skewed toward the higher end of the distribution of iron stores noted in Figure 1, we compared the frequency of mutations for women and men in the upper quartile (Q4) with the combined lower 3 quartiles (Q1-Q3) of iron stores. As shown in Figure 2, 38.1% (16 of 42 women) with iron stores in the upper quartile (>961 mg) had a mutation and this value was not statistically different from 32.8% (41 of 125) of women who had a mutation and had iron stores in the lower 3 quartiles (<961 mg). For men, 53.6% (15 of 28) with iron stores in the upper quartile (>1,050 mg) had a mutation compared to 30.1% (25 of 83)

of men who had a mutation and had iron stores in the lower 3 quartiles (<1,050 mg). This difference was statistically significant, $p < 0.025$. Another way of expressing this difference is that a man with a mutation is 2.7 times (95% CI=1.12-6.54) more likely to have iron stores greater than >1,050 mg than if he had no mutation. None of the following confounding variables, total iron intake, alcohol consumption and inflammatory processes proved significant in explaining why men with a mutation have increased iron stores. Of the 15 men with a mutation, 7 were heterozygous for the 845A mutation with mean iron stores of $1,300 \pm 127$ mg, 7 were heterozygous for the 187G mutation with mean iron stores of $1,233 \pm 165$ mg and 1 was a compound heterozygote (845A/187G) with iron stores of 1,439 mg.

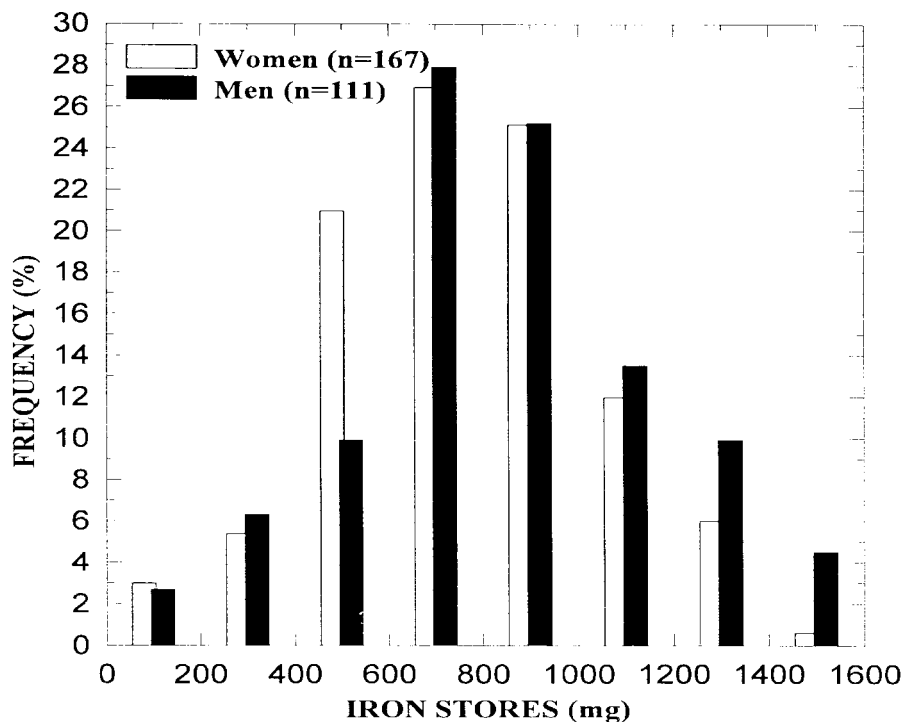


Figure 1. Total n=278. Nine individuals with MCV >100 or <85 excluded.

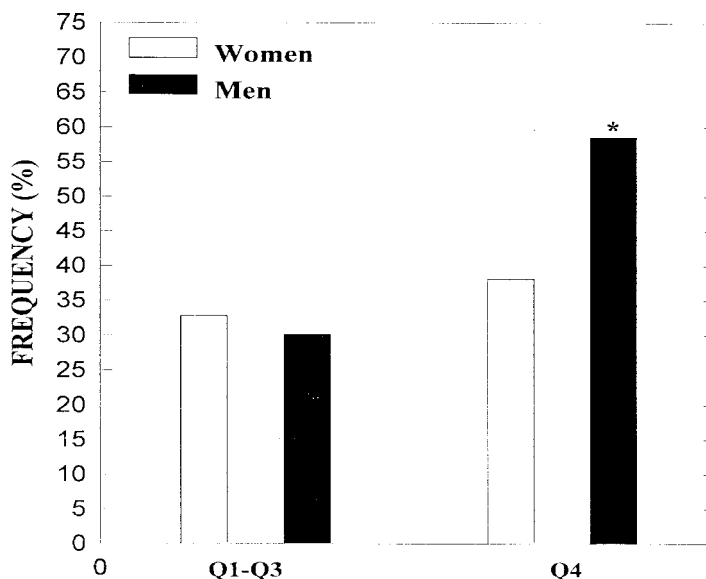


Figure 2. Frequency of mutations in upper quartile (Q4) compared with those in the first 3 quartiles (Q1-Q3) of iron stores. Statistically significant, $p < 0.025$ (Q4 vs Q1-Q3).

DISCUSSION

The discovery of a candidate gene for hemochromatosis has raised an interesting question relative to whether or not individuals found to carry the 845 and/or the 187 mutations are associated with increased PSAT's that might result in increased iron stores, compared to those with no mutations. This issue may be important because of reported associations between high body iron stores and disturbed glucose homeostasis (13), as well as recent epidemiological observations showing that even modest increases in iron stores may increase the risk for ischemic heart disease and certain types of cancer (14-16).

The healthy elderly volunteers enrolled in the NMAPS represent an ideal group in which to examine the association between iron stores and genetic mutations for the following reasons. Bulaj et al. (17) found that the geometric mean serum ferritin concentration in male heterozygotes for HH rose gradually until the sixth decade and declined slightly thereafter while in heterozygous women, the geometric

mean serum ferritin concentration rose until the seventh decade of life. This finding suggests that age is not a confounding factor when examining iron stores and agrees with our finding that iron stores in elderly men and women enrolled in the NMAPS reach steady state levels, since iron stores remain relatively constant for a period up to 10 years even though there is a high degree of within-subject variation in iron stores, as estimated by yearly measurement of serum ferritin concentrations (18,19). In order to decrease the within-subject coefficient of variation and thereby more accurately estimate steady state percent transferrin saturations, as determined by serum iron and TIBC concentrations, and ferritin concentrations, we used means of 4 yearly measurements for each analyte per individual in this study. As noted in Figure 1, iron stores in these elderly women and men vary widely, from less than 100 mg to slightly over 1500 mg. In order to address the question of whether iron stores are influenced by HLA-H mutations, regardless of genotype, we compared mean PSAT's and iron stores for those with the usual genotype to those with any

mutation. Both women and men with any HLA-H mutation had significantly higher mean PSAT's ($p < 0.001$) compared to those with the usual genotype. This finding is in agreement with that reported by Bulaj et al. (17) who found that mean PSAT's were significantly higher in 1,058 heterozygotes for the HLA-linked hemochromatosis mutation than in 321 normal subjects in all age groups studied (1 to 90 years of age) and did not increase with age.

While mean estimated iron stores for both men and women within a particular HLA-H mutation tended to be higher than found in men and women with the usual genotype, we were not able to find significant differences for any of the genotypes when compared to those with the usual genotype when examined by gender. This non-significant finding could be related to the small number of subjects within each genotype cell. When we examined women and men with any mutation, compared to their counterparts with the usual genotype, we found that only men had significantly higher estimated iron stores than did men with the usual genotype, 916 ± 334 and 776 ± 300 mg, respectively, ($p = 0.025$). In order to determine whether men with a mutation had estimated iron stores that were in the upper range of the iron stores distribution, we dichotomized estimated iron stores into two groups, those with estimated iron stores $> 1,050$ mg, (upper quartile, Q4) and those with iron stores $< 1,050$ mg ($< Q4$). As noted in Figure 2, 53.6% of the men with estimated iron stores $> 1,050$ mg had a mutation compared to only 30.1% who had no mutations and had estimated iron stores of less than 1,050 mg. In other words, men with a HLA-H mutation were 2.7 more likely to have increased iron stores compared to men with no mutation. While this was a statistically significant finding, there are confounding variables that could explain these results, such as differences in iron and alcohol intake and inflammatory processes. No confounding variables could explain differences noted between those with a mutation and those with the usual genotype.

An interesting question is why women do not

show the same effect for iron stores as men. As is well known, women are protected prior to menopause from age-related increases in iron stores by menstrual blood losses. However, after menopause iron stores increase in women and approach those for men. It could be that not enough time has elapsed after menopause for women with a HLA-H mutation to increase their iron stores to the point that they would be significantly different from those with the usual genotype. Also, another confounding variable could be related to a certain percentage of women with a mutation who continue on hormone replacement therapy after menopause. We did not examine for this possibility in the present study. It should be noted that this study, examining the effects of iron intake on iron stores, looked at a short interval in the total life history of this group of women and men. Obviously, iron stores measured during this late stage in life reflects a total lifetime history of iron intake and it is not possible to determine whether these individuals received less than adequate, marginal or more than adequate iron intakes to replace obligatory losses during various periods of their life which could delay or accelerate their reaching steady state levels of iron stores relative to the age at which they were examined in this study.

We have concluded from this study that men having either a single chromosomal 845A and/or 187G mutation results in higher PSAT's and iron stores than if no mutation were present. Even though the potential role of the 187G mutation in the phenotypic expression of HH is less certain than the 845A mutation, the increase in PSAT seen in individuals with the 187G mutation and the equal distribution of 845A and 187G mutations seen in men with iron stores $> 1,050$ mg in this study lends support for the involvement of the 187G mutation, or a linked mutation, in iron regulation. Even though progressive disease due to iron overload alone in heterozygous individuals for HH is reported to be rare (17), the interpretation of data relating modest increases in iron stores, due to a HLA-H mutation, to cancer and heart disease remains

controversial. However, it would seem prudent to avoid consuming iron supplements if an individual is known to have a HLA-H mutation.

The gene frequencies for the 845A and 187G mutations in this study were 0.061 and 0.136, respectively, and were lower than reported by Beutler et al. (2), 0.075 and 0.158, respectively, and Barton et al. (3), 0.077 and 0.144, respectively. The Hardy-Weinberg equilibrium would predict that in the general white population, located in and around Albuquerque, NM, the frequency of 845A/845A homozygotes would be 1 out of every 270 individuals. This value is somewhat lower than that reported by Beutler et al. (2), and Barton et al. (3), 1 out of 179 and 1 out of 167, respectively. Based on the gene frequency of the 187G mutation, we predicted that 1 out of every 54 subjects while Beutler et al. (2) predicted that 1 out of every 40 individuals and Barton et al. (3) predicted that 1 out of every 48 individuals should be homozygous for the 187G mutation. Ethnic differences in these three populations no doubt explain some of the variation between the studies.

Finally, Beutler et al. (2) found that the penetrance for the compound heterozygous genotype (845A/187G) was on the order of 1.5% and Feder et al. (1) determined it to be even lower, i.e., 0.5% of expected. Beutler et al. (2) contend that compound heterozygosity likely results in a much milder disease than homozygosity for the 845A mutation. Our data support this. While the 7 subjects found to be compound heterozygous (3 women and 4 men) had significantly higher PSAT, $p < 0.001$, ($41.7 \pm 9.9\%$) and higher estimated iron stores, $p = 0.04$, (991 ± 394 mg) than noted for PSAT and estimated iron stores in the 181 subjects with the usual genotype, i.e., $28.0 \pm 7.4\%$ and 756 ± 290 mg, respectively, these levels of iron stores are of a magnitude lower than usually found in individuals clinically diagnosed with HH. Of interest was the finding that the 7 individuals who were homozygous for the 187G mutation had significantly higher ($p < 0.03$) PSAT's ($33.6 \pm 4.3\%$) but not statistically higher

estimated iron stores (863 ± 225 mg) than those with the usual genotype. The non-significant difference in estimated iron stores noted for those homozygous for the 187 mutation compared to those with the usual genotype can partially be explained by the fact that one male homozygous for the 187 mutation was a regular blood donor during the time period of the study. This may also explain the low level of mean iron stores for the 187G/187G men in Table 3. The final impact of the 187 mutation on iron regulation needs further study.

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