

## Commentary on *HFE* S65C Variant Is Not Associated with Increased Transferrin Saturation in Voluntary Blood Donors by Naveen Arya, Subrata Chakrabarti, Robert A. Hegele, Paul C. Adams

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We had expected that when the *HLA*-linked gene that caused hereditary hemochromatosis was finally identified several different mutations would be found in patients. The linkage disequilibrium with *HLA-A* and *HLA-B* made it likely that there would be one predominant mutation. But by analogy with other single-gene mutations such as those that cause Gaucher disease, Tay-Sachs disease, cystic fibrosis, pyruvate kinase deficiency and many others, several other less prevalent mutations, often existing in different haplotypes, were to be expected. It was surprising, then, that when the *HFE* gene was identified in 1996 patients with hereditary hemochromatosis were found to have only two mutations, the phenotypically severe c.845 G→A (C282Y) and the much milder c.187 C→G (H63D) mutations. Our attempts (1) and those of others (2) failed to find additional mutations in patients with hemochromatosis who either lacked the C282Y or H63D mutations or were simple heterozygotes for one of them. In 1997 another polymorphism involving the hemochromatosis gene was described at an international meeting (3), but this polymorphism occurred at relatively low frequency and its relationship to the disease process was unknown. Recently, Mura et al. reported that the prevalence of this c.193A→T (C65S)<sup>3</sup> mutation was significantly increased in patients with hereditary hemochromatosis with at least one chromosome without an assigned mutation. Among 31 patients with hemochroma-

tosis who were heterozygous for the C282Y mutation, they found five (16%) who carried the S65C mutation. Of 27 patients who appeared to be simple heterozygotes for the H63D mutation, two were found to carry the new mutant allele, and of 35 patients who carried neither of the two other mutant alleles, three carried the S65C allele. Compared with controls, these results were significant with  $p < 0.01$ .

In the paper preceding this commentary, Arya and associates (5) have taken a different approach. They performed a nonparametric analysis of the occurrence of the S65C mutation in a group of first-time blood donors with transferrin saturations of over 45%. After excluding patients carrying the C282Y allele, they found that the group with high transferrin saturations was significantly enriched with the H63D mutation compared with the group with low transferrin saturations. No such enrichment for the S65C mutation could be demonstrated.

It has been difficult to obtain robust data about the effect of the S65C mutation because of its low frequency in the population. While gene frequency for the C282Y mutation is approximately 0.07 and that of the H63D mutation about 0.16 in northern European populations, the frequency of the S65C mutation is only 0.015. If the effect of the S65C mutation were a very modest one, it is likely that a large number of subjects would be required to detect it. Moreover, it may be that its effect would

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<sup>3</sup>To avoid confusion we have adopted the same nomenclature used by Arya et al, designating the 187G, 193T, and 845A mutations as H63D, C65S, and C282Y, and the haplotypes in which they occur as D63, S65, and Y282. Ordinarily we favor the more robust nucleotide-based designation of mutations and haplotypes, particularly since alternative amino acid numbering schemes have been used (4). The designation *wt* for wild type is used to designate the allele that contains no mutations.

only become evident if another mutant gene were in the *trans* position, as was the case in many of the patients reported by Mura et al (6).

We have undertaken large-scale screening of patients being examined at the Kaiser Permanente San Diego Health Appraisal Clinic and have thus far performed genotypes on 9179 patients on whom transferrin saturation values were obtained. We were stimulated by the conflicting results about the S65C that have been reported by Mura et al. (6) and Arya et al. (5) to make a detailed analysis of the data collected in this ongoing investigation. Some of the results that we have obtained from these patients are summarized in Tables 1 and 2. In each case we compare the transferrin saturation of a group of patients who share one haplotype, but in which the other haplotype is either S65C or *wt*. If we perform a nonparametric analysis, i.e. compare the frequency of the S65C mutation in patients with transferrin saturations equal to or over 45% with those with transferrin saturations under 45% we obtain results very similar to those reported by Arya et al.; there is no significant difference between groups with and without the S65C mutation. Our results also confirm their observation that the H63D mutation is associated with an increase in the

**Table 1.** The Effect of Heterozygosity for the S65C Mutation with the Wild Type (*wt*) and the H63D and C282Y HFE Mutations on Transferrin Saturation (TS): Non-parametric Analysis

Genotype	TS = >45 %	TS < 45%	% = > 45%
Male			
<i>wt/wt</i>	109	2612	4.01
C65/ <i>wt</i>	4	81	4.71
D63/ <i>wt</i>	52	864	5.68
D63/C65	4	18	18.2
Y282/ <i>wt</i>	25	316	7.33
Y282/C65	1	8	11.11
Female			
<i>wt/wt</i>	50	2856	1.72
C65/ <i>wt</i>	2	79	2.47
D63/ <i>wt</i>	27	933	2.81
D63/C65	0	18	0
Y282/ <i>wt</i>	21	349	5.68
Y282/C65	0	7	0

**Table 2.** The Effect of Heterozygosity for the S65C Mutation with the Wild Type (*wt*) and the H63D and C282Y HFE Mutations on Transferrin Saturation (TS): Parametric Analysis

Genotype	n	Mean TS	Standard deviation	Difference t; p
Male				
<i>wt/wt</i>	2721	26.32	9.33	+1.26
C65/ <i>wt</i>	85	27.58	9.18	1.25; NS
D63/ <i>wt</i>	916	28.86	9.57	+ 4.78
D63/C65	22	33.64	10.76	2.06; 0.05
Y282/ <i>wt</i>	341	30.33	9.54	+ 3.34
Y282/C65	9	33.67	10.69	0.9; NS
Female				
<i>wt/wt</i>	2906	22.26	8.96	+ 2.64
C65/ <i>wt</i>	81	24.90	9.51	2.46; 0.05
D63/ <i>wt</i>	960	24.50	9.39	+ 0.67
D63/C65	18	25.17	9.16	0.3; NS
Y282/ <i>wt</i>	370	26.84	9.70	+ 6.73
Y282/C65	7	33.57	9.16	1.92; 0.05

Note. t is Students t.

percentage of subjects with transferrin saturations of equal to or over 45%. However, a parametric analysis (Table 2) shows that in every instance, comparison of genotypes in which the *wt* or the S65C allele is in *trans* position from any other allele the mean transferrin saturation is higher in heterozygotes for the S65C mutation. Some of the individual values reach a significance level of  $p = 0.05$ ; collectively, the unweighted sum of the t values gives  $z = 3.63$ ,  $p = .00014$  and the weighted sum gives  $z = 3.25$ ,  $p = 0.00058$  (7). Accordingly, the data indicate that the S65C mutation does have an effect on iron metabolism. Perhaps this is not too surprising, since it seems to be in a region that has been implicated in the binding of transferrin receptor to the HFE protein (4).

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