**Letters to the Editor**

**Transferrin Saturation and Mortality**

*To the Editor:*

Ellervik and colleagues (1) recently reported a positive association between transferrin saturation (TS) and mortality. Several questions arise from this observation: Is the association due to all causes of iron overload or to hereditary hemochromatosis only? Does the study underestimate the true association? And, is mortality due to variation in iron, transferrin, or both?

We have relevant data from population-based studies of twins and families of European descent living in Australia (2, 3). TS values (calculated from serum transferrin and iron) and *HFE* (hemochromatosis) genotypes for C282Y (rs1800562, genotyped) and H63D (rs1799945, imputed) are available for 8096 adults (3151 men and 4945 women; mean age, 47 years). Replicate TS measurements are available for 460 participants (178 men and 282 women) from studies in 1993–1996 and 2001–2005. Their mean age at the time of the second study was 50 years (range, 39–72 years).

The Discussion in the Ellervik et al. report implies that the association of TS with mortality is driven by the C282Y variant (which is associated with hemochromatosis) and that TS is acting as a surrogate for this variant. There is a lack of equivalence between TS values >50% and *HFE* variants, however. Table 1 shows the relationships between TS and genotype for the 288 participants for whom TS values were ≥50% and both genotypes were available. Although just over half of the people with the YY genotype have a TS value ≥50%, only a minority of people with a TS value ≥50% are YY (about 10% for men and 20% for women). About a third of those with an increased TS are homozygous for the common allele. The inference that the relationship between TS and mortality is due to clinically unexpressed hemochromatosis needs further support before it can be accepted; high iron availability from any cause may be harmful. The situation is further complicated by the multiple effects of the 282Y allele, which not only increases TS but also decreases LDL cholesterol (4). Thus, this variant could produce adverse effects due to higher iron concentrations and beneficial effects due to lower LDL concentrations.

Second, an increased TS may contribute more to total mortality than this study indicates. As in many prospective studies, the predictor variable was measured only once; consequently, no allowance was made for the regression dilution effect caused by analytical and biological variation. As these authors point out, measurement errors and within-person variation in a risk factor study will produce a bias toward the null—that is, the size of the effect is underestimated. The degree of underestimation can be assessed from measurements taken on 2 occasions, either on the original cohort or with data from comparable groups. We found a significant but low 9-year repeatability for TS: \( r = 0.30 \) (\( n = 178; P = <0.0001; 95\% \text{ CI}, 0.16–0.43 \)) for men, and \( r = 0.33 \) (\( n = 282; P = <0.0001; 95\% \text{ CI}, 0.23–0.43 \)) for women. Therefore, the necessary adjustment [\( \lambda = 1/r(5) \)] is approximately 3. The concordance for high TS values was poor, because only 3 people had TS values ≥50% on both occasions. An assessment of the true odds ratio is complicated by the binary classification of the TS results, but the effect of the long-term mean TS on mortality will be substantially greater than the initial estimate—possibly 3 times as great.

Third, TS is a derived variable, so the association with mortality may be with iron, transferrin, or possibly both, but in opposite directions. It would be interesting if the authors could reanalyze their data to test whether iron and transferrin are predictors of mortality.

Our data and conclusions are subject to some limitations. There are differences between Australia and Denmark that have the potential to affect the relationships between high TS and *HFE* genotypes and/or between high TS and the repeatability of TS (and therefore the regression dilution factor). Additionally, how the regression dilution factor applies to dichotomous data is not clear, although it is applicable to survival and logistic re-

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**Table 1. Percentages of individuals with TS values ≥50% by C282Y and H63D genotype, and of those with TS values ≥50% having each genotype.**

<table>
<thead>
<tr>
<th>HFE genotype</th>
<th>Individuals with TS values ≥50% (by genotype), % (n)</th>
<th>Contribution of each genotype to the group with TS values ≥50%, % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YY/HH</td>
<td>Male 64.0 (16/25) Female 53.5 (23/43)</td>
<td>Male 9.4 (16/171) Female 19.7 (23/117)</td>
</tr>
<tr>
<td>CY/HD</td>
<td>Male 31.1 (23/74) Female 13.3 (16/120)</td>
<td>Male 13.5 (23/117) Female 13.7 (16/117)</td>
</tr>
<tr>
<td>CC/DD</td>
<td>Male 11.0 (8/73) Female 4.0 (4/101)</td>
<td>Male 4.7 (8/171) Female 3.4 (4/117)</td>
</tr>
<tr>
<td>CY/HH</td>
<td>Male 5.9 (22/370) Female 2.9 (16/561)</td>
<td>Male 12.9 (22/171) Female 13.7 (16/117)</td>
</tr>
<tr>
<td>CC/HD</td>
<td>Male 5.6 (39/696) Female 2.0 (22/1109)</td>
<td>Male 22.8 (39/171) Female 18.8 (22/117)</td>
</tr>
<tr>
<td>CC/HH</td>
<td>Male 3.3 (63/1913) Female 1.2 (36/3011)</td>
<td>Male 36.8 (63/171) Female 30.8 (36/117)</td>
</tr>
</tbody>
</table>

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**Footnotes:**

1. Ellervik and colleagues (1) recently reported a positive association between transferrin saturation (TS) and mortality. Several questions arise from this observation: Is the association due to all causes of iron overload or to hereditary hemochromatosis only? Does the study underestimate the true association? And, is mortality due to variation in iron, transferrin, or both?

2. *HFE* (hemochromatosis) genotypes for C282Y (rs1800562, genotyped) and H63D (rs1799945, imputed) are available for 8096 adults (3151 men and 4945 women; mean age, 47 years).


4. Their mean age at the time of the second study was 50 years (range, 39–72 years).

5. The Discussion in the Ellervik et al. report implies that the association of TS with mortality is driven by the C282Y variant (which is associated with hemochromatosis) and that TS is acting as a surrogate for this variant. There is a lack of equivalence between TS values >50% and *HFE* variants, however. Table 1 shows the relationships between TS and genotype for the 288 participants for whom TS values were ≥50% and both genotypes were available. Although just over half of the people with the YY genotype have a TS value ≥50%, only a minority of people with a TS value ≥50% are YY (about 10% for men and 20% for women). About a third of those with an increased TS are homozygous for the common allele. The inference that the relationship between TS and mortality is due to clinically unexpressed hemochromatosis needs further support before it can be accepted; high iron availability from any cause may be harmful. The situation is further complicated by the multiple effects of the 282Y allele, which not only increases TS but also decreases LDL cholesterol. Thus, this variant could produce adverse effects due to higher iron concentrations and beneficial effects due to lower LDL concentrations.

6. Second, an increased TS may contribute more to total mortality than this study indicates. As in many prospective studies, the predictor variable was measured only once; consequently, no allowance was made for the regression dilution effect caused by analytical and biological variation. As these authors point out, measurement errors and within-person variation in a risk factor study will produce a bias toward the null—that is, the size of the effect is underestimated. The degree of underestimation can be assessed from measurements taken on 2 occasions, either on the original cohort or with data from comparable groups. We found a significant but low 9-year repeatability for TS: \( r = 0.30 \) (\( n = 178; P = <0.0001; 95\% \text{ CI}, 0.16–0.43 \)) for men, and \( r = 0.33 \) (\( n = 282; P = <0.0001; 95\% \text{ CI}, 0.23–0.43 \)) for women. Therefore, the necessary adjustment [\( \lambda = 1/r(5) \)] is approximately 3. The concordance for high TS values was poor, because only 3 people had TS values ≥50% on both occasions. An assessment of the true odds ratio is complicated by the binary classification of the TS results, but the effect of the long-term mean TS on mortality will be substantially greater than the initial estimate—possibly 3 times as great.

7. Third, TS is a derived variable, so the association with mortality may be with iron, transferrin, or possibly both, but in opposite directions. It would be interesting if the authors could reanalyze their data to test whether iron and transferrin are predictors of mortality.

8. Our data and conclusions are subject to some limitations. There are differences between Australia and Denmark that have the potential to affect the relationships between high TS and *HFE* genotypes and/or between high TS and the repeatability of TS (and therefore the regression dilution factor). Additionally, how the regression dilution factor applies to dichotomous data is not clear, although it is applicable to survival and logistic re-
gression as well as to bivariate quantitative data (5).

The points made with respect to TS apply to other existing or proposed risk factors. The choice between quantitative measurements and genotyping is important and will have to be made on a case-by-case basis as our knowledge of the effects of single-nucleotide polymorphisms on risk and risk factors increases. Quantitative risk factors are subject to analytical and biological variation, which can lead to underestimation of their contributions to risk. Methods exist to adjust for underestimation at an epidemiologic level, but they may not improve the detection of high-risk patients.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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