will enhance their appeal and universality without compromising the importance of MIQE as a set of standards that is beginning to achieve acceptance in the scientific community.

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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

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

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

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% CI, 0.16–0.43) for men, and $r = 0.33$ (n = 282; $P = <0.0001$; 95% 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 poten-

tial to affect either the relationships between high TS and *HFE* genotypes or 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 regression 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.

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In Reply

We thank Benyamin et al. for their response to our recent article (1). We are puzzled by their statement that our Discussion implies that the association of total mortality with increased transferrin saturation is driven by the C282Y/C282Y genotype. We intentionally did not write about genetic hemochromatosis in our paper; rather, our referral to "hemochromatosis" was meant to imply hemochromatosis by any cause, genetic or otherwise. The information regarding hemochromatosis genotypes, however, is available for the participants in both the Copenhagen City Heart Study and the Copenhagen General Population Study (2, 3). We have now calculated total mortality risks for both studies combined, as a function of hemochromatosis genotype vs wild type/wild type [design and statistics as described (1)]. These risks are: 1.2 (95% CI, 1.0–1.3) for C282Y/wild type; 0.8 (95% CI, 0.6–1.1) for C282Y/H63D; and 1.3 (95% CI, 0.7–2.4) for C282Y/C282Y. Thus, the increased association of total mortality with increased transferrin saturation that

we noted was not driven mainly by hemochromatosis genotype.

We fully agree with the statement of Benyamin et al. regarding regression dilution bias (4) and originally considered correcting our results for this bias. For that purpose, we used measurements of transferrin saturation in the Copenhagen City Heart Study that we obtained for 4302 individuals on 2 examinations 10 years apart (1991–1994 and 2001–2003). The regression dilution factor we obtained (2.7) was used to correct total-mortality hazard ratios for transferrin saturation levels ≥50% vs levels <50%. We compared the corrected results with the uncorrected results from Table 2 in (1) for all sexes [corrected, 2.3 (95% CI, 1.5–3.7); uncorrected, 1.4 (95% CI, 1.2–1.6)], for men [corrected, 2.4 (95% CI, 1.4–4.7); uncorrected, 1.3 (95% CI, 1.1–1.6)], for women [corrected, 3.0 (95% CI, 1.7–6.5); uncorrected, 1.5 (95% CI, 1.1–2.0)], for the Copenhagen General Population Study [corrected, 4.9 (95% CI, 1.6–13); uncorrected, 1.8 (95% CI, 1.2–2.6)], and for the Copenhagen City Heart Study [corrected, 1.6 (95% CI, 1.0–2.5); uncorrected, 1.2 (95% CI, 1.0–1.4)].
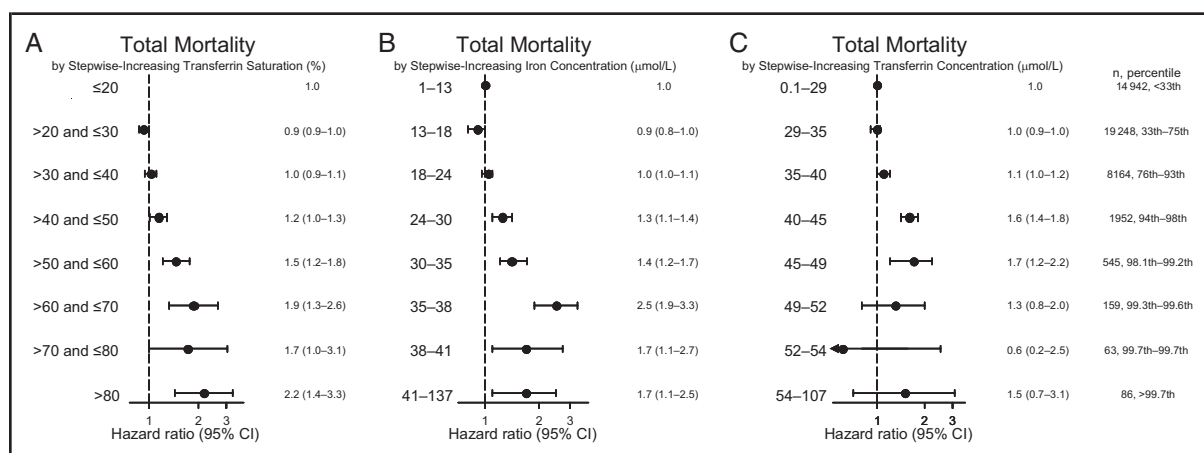
Fig. 1. Total mortality by stepwise increases in transferrin saturation (A), iron concentration (B), and transferrin concentration (C) in individuals in the general population.

Results are shown for the Copenhagen General Population Study and the Copenhagen City Heart Study combined. Results for iron and transferrin concentration are shown by stepwise-increasing percentiles, similarly to transferrin saturation.