

Commentary on Significance of Linkage Disequilibrium between Mutation C282Y and a *MseI* Polymorphism in Population Screening and DNA Diagnosis of Hemochromatosis by J. Nico P. de Villiers and Maritha J. Kotze

The polymerase chain reaction (PCR) has revolutionized the practice of molecular biology and has made possible the identification of mutations in large populations. The implementation of PCR for this purpose seems straightforward and because the procedure is so deceptively simple possible pitfalls are often forgotten. If only one of two different alleles is amplified, the PCR product is homogeneous and is indistinguishable from the product observed when the DNA from a homozygote is amplified. The existence of this potential problem has been known for a long time. It has given rise to inaccurate diagnoses, for example, in cystic fibrosis and Gaucher disease (1,2). Now it is apparent that this possibility also exists in diagnosis of the 845A (C282Y) mutation of hereditary hemochromatosis. One of the not-too-surprising findings in this disorder has been that there are asymptomatic individuals, even elderly ones, who have been reported to be homozygous for this mutation (3-6). Now the question arises whether these individuals were actually homozygotes. As de Villiers et al. point out, whether or not compound heterozygotes with the 845A and the 5569G→A mutation will be misidentified as homozygotes for the 845A mutation depends greatly on the conditions of PCR, and these are not always explicitly stated.

We are in the process of performing a population survey of patients attending a health appraisal clinic and have thus far examined 6298 individuals over the age of 26. Thirty-three of these were diagnosed as homozygotes for the 845A mutation. Because our PCR conditions were such as to allow the downstream primer to anneal even in the presence of the 5569G→A mutation, all of these diagnoses proved to be correct and could be validated with a second set

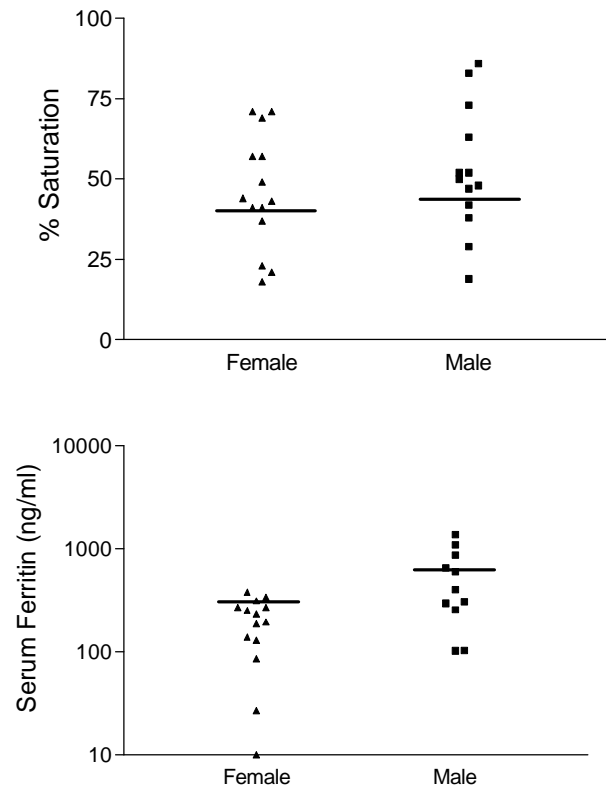


Figure 1. The serum ferritin levels and transferrin saturations of subjects with the 845A/845A (C282Y/C282Y) genotype detected in a screening program in a health appraisal clinic. In the entire patient population the transferrin saturation for females was 22.75 ± 9.53 (mean \pm S.D.) and of males 26.5 ± 9.37 . The ferritin level of females averaged 57.9 ng/ml (log average ferritin = 1.7629; S.D. log ferritin = 0.37133). The ferritin level of males averaged 125.5 ng/ml (log average ferritin = 2.099; S.D. log ferritin = 0.36149). The horizontal lines represent 2 standard deviations above the arithmetic mean of the transferrin saturation and of the geometric mean of the ferritin level.

of primers that did not include the mutation. Six of these patients had previously been diagnosed with hereditary hemochromatosis and started on a phlebotomy program. Figure 1 summarizes the transferrin saturation and serum ferritin levels of all of the other 27 subjects in which they were

available. Most of these patients were asymptomatic and many of them had normal iron studies. Indeed, only two-thirds had transferrin saturations more than two standard deviations above the mean and if a conservative 50% saturation were used as a cutoff only about one-half would have been detected by biochemical screening.

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