

Significance of Linkage Disequilibrium between Mutation C282Y and a *MseI* Polymorphism in Population Screening and DNA Diagnosis of Hemochromatosis

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Abstract: An increasing number of studies demonstrate a lack of phenotypic expression in subjects found to be homozygous for the common hereditary hemochromatosis (HH) mutation, C282Y. In this study the impact of possible overestimation of C282Y homozygosity, as a consequence of a *MseI* polymorphism identified in intron 4 of the HFE gene, was investigated in South African subjects. Utilization of a modified polymerase chain reaction (PCR)-based assay highlighted the potential implications with respect to genotype/phenotype correlation studies, particularly in the general population. Mistyping rather than lack of disease association provides a plausible explanation for the phenomenon of C282Y homozygosity without iron overload. Reassessment of C282Y mutation status in such cases may result in justified population screening in HH.

Hereditary hemochromatosis (HH) is considered to be the most common (1/200–400) autosomal recessive disorder in individuals of northern European descent. This disease is associated with myocardopathy, cirrhosis, endocrine dysfunction and diabetes mellitus, due to organ damage as a result of progressive iron overload. A single missense mutation C282Y in the HFE gene is responsible for HH in approximately 80% of all affected cases worldwide (1,2). Although several different methods have been developed for detection of this mutation, most analyses are based on polymerase chain reaction (PCR) amplification of a 389-bp fragment using the oligonucleotide primers described by Feder et al (1). However, a *MseI* polymorphism has recently been identified in intron 4 (IVS4+48G→A) of the HFE gene (3), within the binding area of the antisense primer commonly used for C282Y mutation screening. Jeffrey et al (4) reported that this variant (designated 5569G/A) might cause overestimation of C282Y homozygote prevalence in hemochromatosis, due to linkage disequilibrium with mutation C282Y. We have investigated this aspect in the South African population.

C282Y mutation screening in Afrikaners of European ancestry confirmed the high prevalence of HH (5) in this population, and led to the implementation of a molecular diagnostic service for HH in South Africa (6). During the past two years, 65 of the subjects referred for molecular diagnosis (or confirmation) of HH on the basis of abnormal iron profiles were found to be homozygous for the C282Y mutation. Re-amplification of these DNA samples, using the new antisense primer designed by Jeffrey et al (4) excluding the *MseI* polymorphic site, confirmed C282Y homozygosity in this selected group of 65 patients. However, preferential amplification of the mutant C282Y allele due to the presence of the intron 4 variant on the normal chromosome, was demonstrated by *RsaI* and *MseI* restriction enzyme analysis (Fig. 1) in a number of individuals identified as putative homozygotes following population screening. These included a putative C282Y homozygote identified among 200 elderly individuals (>70 years), and one of 83 patients with familial hypercholesterolemia (FH), recruited for future genotype/phenotype correlation studies. Since band intensity after ethidium bromide staining decreases with smaller DNA fragment size, the faint 111-bp fragment

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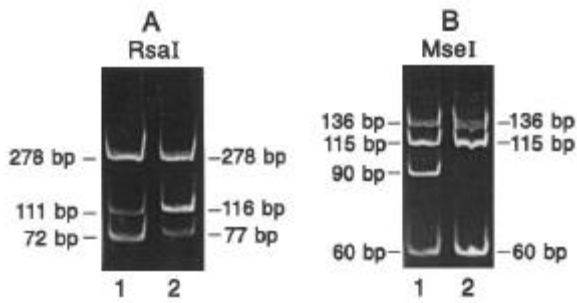


Figure 1. Restriction enzyme analysis of PCR-amplified DNA from a putative C282Y homozygote. The polyacrylamide (PAA) gels were stained with ethidium bromide and the DNA fragments visualized under ultraviolet light. (A) Gel electrophoresis (12% PAA, 3.4% C) of *RsaI* digested genomic DNA amplified with the original primer set (lane 1) and with the new anti-sense primer that excludes the site of the *MseI* polymorphism (lane 2). (B) Gel electrophoresis (10% PAA, 5% C) of *MseI* digested genomic DNA (lane 1) amplified with PCR primers corresponding to exon 4 (5' GTATGTGACTGATGACAGCCA 3') and intron 5 (5' CAGAGGTAAGAGACTTC 3') sequences. The PCR product of a control individual without the *MseI* polymorphism was loaded in lane 2.

observed in these cases was initially ascribed to partial *RsaI* digestion and the subjects labeled as putative C282Y homozygotes. The *MseI* polymorphism was not expected to disrupt annealing of the antisense primer on the normal chromosome in C282Y heterozygotes, since PCR primers are often designed with single or double mismatches to improve specificity or to incorporate a new restriction enzyme recognition site for cloning purposes. The PCR cycling conditions appeared to be a very important factor, because lowering of the annealing temperature from the previously used 60 C to 55 C, resulted in equal amplification of both alleles when using the primers designed by Feder et al (1), irrespective of the presence or absence of the intron 4 variant in the subjects analyzed (data not shown).

Accurate determination of C282Y status is of particular relevance in the South African context, where the high carrier frequency (1/6) of this mutation in Caucasians (6) may have important implications not only for patients with HH, but also for FH or variegate porphyria (VP) sufferers. The high prevalence of FH and VP (7,8), considered to be the most common autosomal

dominant diseases in South Africa, is due to founder gene mutations in the inbred Afrikaner population (9,10). This situation provides a valuable research tool that is being explored to investigate the significance of the lower C282Y mutation frequency detected in Afrikaners with VP compared with controls (3). Preliminary data also revealed allelic differences in molecularly characterized Afrikaner FH patients, which may be related to increased atherosclerosis risk (11,12) in FH patients with the C282Y mutation. In genetic studies such as these involving clinical correlations, it is of utmost importance that possible confounding factors be excluded.

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