Efficient Large-Scale Screening for the Hemochromatosis Susceptibility Gene Mutation

To the Editor:

Hereditary hemochromatosis (HH) is an autosomal-recessive iron storage disease associated with widespread tissue injury including cirrhosis, endocrine failure, joint inflammation, and cardiac disorders. HLA-H has recently been reported to be a candidate gene for HH on the short arm of chromosome 6, as two separate mutations have been found in HH patients. Eighty-seven percent of HH patients have a G to A transition at nucleotide 845 of the open reading frame of HLA-H that results in a cysteine to tyrosine substitution at amino acid 282 (C282Y).1 Subsequently, other groups have confirmed the finding and demonstrated this missense mutation in 90% and 100% of HH patients, respectively.2,3 The implications of the second missense variant H63D caused by a C to G transversion at 187 nucleotides (nt) of the open reading frame, however, were uncertain as it occurred at a much lower frequency than the C282Y mutation in HH patients and occurred at the same frequency in the general population. The C282Y mutation appears to be closely related to HH but the H63D variant is most likely a polymorphism.2,3 HH is a prevalent disorder and is often diagnosed only after significant organ damage has occurred. Treatment with regular phlebotomy will normally provide effective control of excessive iron stores but damage to organs such as liver and pancreas cannot be reversed by this treatment. Effective prevention of organ damage from HH will require screening of large population groups to identify heterozygotes and homozygotes at risk of the disorder and will only be feasible if reliable and cost-effective methods are available.

As both single base substitutions of the HLA-H gene create or abolish restriction endonuclease recognition sites, polymerase chain reaction (PCR) products spanning the regions of interest were digested with appropriate restriction enzymes for investigating the base substitutions in all previous reports.2-4,6-8 Here we report a more rapid and simple method for large scale screening of the C282Y mutation by allele specific PCR (AS-PCR) combined with facilitated genomic

Fig 1. (A) Strategy for the AS-PCR. Each allele is distinguished by its product size. A common product is amplified from both alleles by the primers F1 and R1. The primer Fm recognizes only the mutant allele sequence while the primer Rw recognizes only the wild-type sequence. *G to A mutation (C282Y) creates a new Rsa I site. (B) Results of 20 subjects screening demonstrating 16 normal subjects, 3 carriers (*) and 1 homozygote (†). C, common product; M, mutant allele; W, wild-type allele; Mk, size marker (Alu I-digested pUC19).
DNA extraction from whole blood. AS-PCR is a multiplex PCR method based on the fact that a successful PCR amplification requires the sequence of 3’ oligonucleotide ends to be absolutely complementary to the DNA template. Figure 1A shows a schematic of the AS-PCR strategy which includes four different oligonucleotide primers consisting of two non–allele-specific primers (Fi: AAGCAGCCAATGGATCCCA and Ri: CCACCTGATGCTTCTCAATGCTA) and two allele-specific primers (Rw: GCCCTGGTGTCCCTACCTGGC, Fm: GGGAAAGCGAGATATAGCTGA). Genomic DNA was prepared by heating 5 μL whole blood in 20 μL of 0.05 N NaOH at 95°C for 20 minutes, followed by neutralization in 20 μL of 0.15 mol/L Tris-HCl (pH 6.5) and 3 μL of the sample was then directly used for the AS-PCR. PCR reaction mixtures consisted of 3 μL of the sample DNA as template, 200 μmol/L of each dNTPs, 200 nmol/L Rw, 1,000 nmol/L Fm, 1,000 nmol/L F1, 1,000 nmol/L R1, and 0.7 U AmpliTaq Gold (Perkin-Elmer, Branchburg, NJ) for the hot start PCR in 20 μL total volume. Conditions for amplification were an initial denaturation at 95°C for 11 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 64°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 10 minutes.

We screened 1,032 unrelated whites in the greater Dunedin area of New Zealand (ethical approval was obtained). Figure 1B shows results obtained from 20 subjects among whom 16 are normal, demonstrating the wild-type allele (W) and common products (C), while 3 are carriers with the wild-type allele, the C282Y mutant allele (M) and common products, and 1 is a homozygote with the mutant allele and common products. One hundred ninety-two heterozygotes and 10 homozygotes for the C282Y mutation were observed among a total of 1,032 subjects. Estimated frequencies for normal and mutant alleles were 0.897 and 0.103, respectively. The mutant allele frequency was one of the highest as yet reported.1,3-8

Evaluation of costs has shown that disposable reagents and supplies cost of the order of $1.37 (US) per sample. The costing was based on testing 90 samples in a batch 96-well process that included 6 hours for testing, interpretation, and reporting per patient with hepatoblastoma.4 Therefore, we assessed serum TPO levels and looked into its biological as well as clinical significance in hepatoblastoma.3,4,7 Therefore, serum TPO levels were assessed in seven untreated patients with hepatoblastoma by using a sandwich enzyme-linked immunosorbent assay (ELISA)3 in addition to IL-1β and IL-6 levels.

High serum TPO levels were observed in all samples examined, ranging from 3.15 to 11.02 fmol/mL (mean ± SD: 6.08 ± 1.25, normal range is 0.33 to 1.72 in adults,5 but not currently available in childhood) (Table 1). Serum IL-1β and IL-6 levels (mean ± SD) in five patients were 0.32 ± 0.08 and 22.9 ± 7.9 pg/mL, respectively (Table 1). IL-6 levels were also higher than normal range. Platelet counts appeared to correlate relatively with serum TPO levels (P < .01), but not with IL-1β and IL-6 levels. Additionally, a serial measurement in a patient indicated that TPO levels decreased along
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