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HFE Gene Mutation, C282Y Causing Hereditary Hemochromatosis in Caucasian is Extremely Rare in Korean Population

Hereditary hemochromastosis (HFE), which affects 1 in 400 and has an estimated carrier frequency of 1 in 10 individuals in Western population, results in multiple organ damage caused by iron deposition, and is treatable if detected early. C282Y mutation in *HFE* gene has been known to be responsible for the most hereditary hemochromatosis cases and 5-10% of white subjects are heterozygous for this mutation. However, the prevalence of hemochromatosis in the Asian population was reported to be very low and ethnic heterogeneity has been suspected. The aim of our study was to determine the prevalence of heterozygosity and homozygosity for the C282Y *HFE* gene mutations in 502 unrelated Koreans. Results revealed that none of them had the mutant gene, suggesting a significant ethnic difference when compared with Caucasians. Our study excluded underlying possibility of hereditary hemochromatosis in Korean which could mimic the findings of alcoholic liver disease with iron overload or liver cirrhosis with chronic hepatitis C.

Key Words: *Hemochromatosis, Genes; Mutation; Point Mutation; Korea*

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INTRODUCTION

Hereditary hemochromatosis (HFE) is an autosomal recessive iron storage disease associated with widespread tissue injury leading to liver cirrhosis, hepatoma, diabetes, cardiomyopathy, arthritis and hypogonadotropic hypogonadism. All these complications can be prevented by phlebotomy if iron excess is detected at a very early stage. *HLA-H* has recently been reported to be a candidate gene for HFE on the short arm of chromosome 6, as two missense mutations have been found in HFE patients (Cys282Tyr & His63Asp). Eighty-five percent of HFE patients have a G to A transition at nucleotide 845 of the open reading frame of *HLA-H*, resulting in a cysteine to tyrosine substitution at amino acid 282 (C282Y) (1). Subsequently, other groups reported even higher rates of homozygous C282Y in their HFE patients [100% (2) and 91% (3), respectively]. The implication of the second missense variant H63D caused by a C to G transversion at 187 nucleotides of the open reading frame, which result in a histidine to aspartic acid substitution at position 63, was uncertain because of its high frequency on control chromosomes (17%) and the relative absence of patients homozygous for this allele (1). Detection of these mutations is certainly of value in diagnosing and screening HFE individuals.

We report here a screening of the C282Y mutation in 502 unrelated Koreans by allele specific polymerase chain reaction (AS-PCR) (4) in order to identify the frequency of this specific mutation and predict the prevalence of hereditary hemochromatosis.

MATERIALS AND METHODS

Genomic DNA was extracted from the blood spots obtained from 502 unrelated normal newborns by neonatal screening tests. Genomic DNA was prepared by heating 10 spots of blood absorbed filter paper in 300 μL of distilled water at 100℃ for 10 min, followed by ethanol precipitation. Then 2 μL of the sample was directly used for the AS-PCR as described (4). PCR reaction

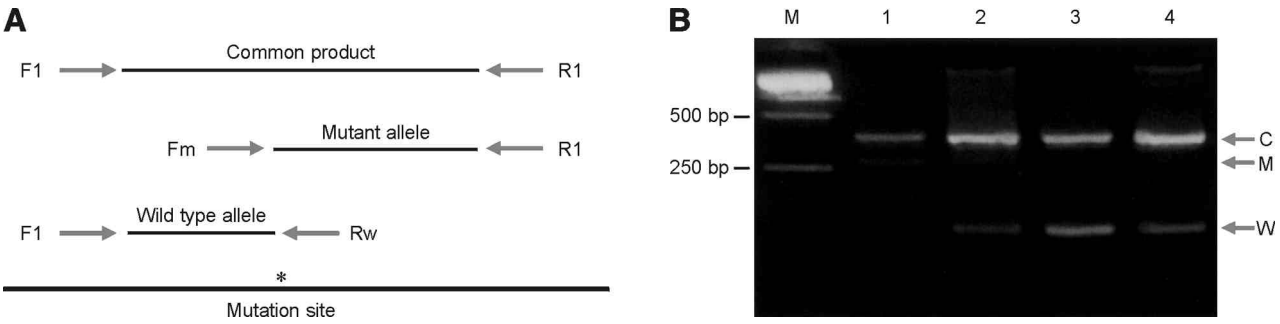

Fig. 1. A: An AS-PCR strategy. **B:** Results of 4 subjects screened showing 3 normal and one homozygote (1). C, common product; M, mutant allele; W, wild-type allele.

mixtures consisted of 2 μL of the sample DNA as template, 200 nmol/L primer-Rw (CCTGGGTGCTCCACCTGGC), 1,000 nmol/L primer-Fm (GGGAAGAGCAGAGATATACGTA), 1,000 nmol/Lprimer-F1 (AAGCAGCCAATGGATGCCAAG), 1,000 nmol/L primer-R1 (CCACTGATGACTCCAATGACTA) and PCR-premix (Bioneer Co. Chungwon, Korea) in 20 μL total volume. Conditions for amplification were an initial denaturation at 95℃ for 5 min followed by 35 cycles of denaturation at 95℃ for 30 seconds, annealing at 62℃ for 30 seconds, and extension at 72℃ for 30 seconds, with a final extension at 72℃ for 10 min. PCR product was run on gel and analyzed. Amplified common product (467 bp) was digested with *Rsa* I restriction enzyme in order to identify the C282Y mutation which creates a new *Rsa* I restriction site. A positive control kindly provided by Dr. Gahl at Heritable Disease Branch, NIH U.S.A. was used for comparison throughout the procedure.

Ninety-five percent confidence intervals were calculated assuming a binomial distribution, and χ^2 analysis was performed using SPSS 8.0 (SPSS Inc, Chicago, IL, U.S.A.).

RESULTS

All the 502 cases were normal, demonstrating the wild-type allele and common product by AS-PCR. As the C282Y mutation creates a new *Rsa* I restriction site, the 467 bp common product digested with *Rsa* I shows two fragments of 278 and 83 bp in normal DNA while three fragments of 249, 83 and 29 bp are generated in mutated DNA (Fig. 1). After digestions of common product with *Rsa* I, all of them showed two fragments of 278 and 83 bps revealing normal genotype. The C282Y allele frequency is significantly lower in Koreans than in Europeans (0.0% vs 3.8%) (5). In conclusion, in 1004 alleles obtained from 502 normal newborns, none of them showed the C282Y mutation, suggesting it is an extremely rare genotype in Korean populations.

DISCUSSION

Hereditary hemochromatosis (HFE) is considered one of the most common genetic diseases of northern European origin with a prevalence estimated at 0.05-0.08 in certain Caucasian populations (6). Feder et al. (1) found that 83% of HFE patients were homozygous for the C282Y mutation. Jawinska et al. (2) found that 100% of Caucasian Australian patients with positive family history of HFE were homozygous for C282Y, and Beutler et al. (7) and Jouanolle et al. (8) found that 82.3% and 92.4% of Caucasian HFE patients in the U.S.A. and Brittany, respectively, were homozygous for C282Y. The distribution of the C282Y mutation coincides with that of populations in which HFE has been reported and is consistent with the theory of it having a northern European origin (5). Because of the high frequency of hereditary hemochromatosis in Caucasian, population-based screening using DNA test has been undertaken (9-11). Subsequent analysis of many different European populations showed similar results as well (12-17). Recent studies (7) suggest the *HFE* gene has been the subject of selective pressure like sickle-cell anemia. This selection pressure could be due to infectious diseases, environmental conditions or other genetic disorders such as anemia.

Of interest, hereditary hemochromatosis has been known to be relatively uncommon in Asian populations and none of the hereditary hemochromatosis cases have been reported in Korea so far. Chang et al. (18) reported that the C282Y mutation was not present in Chinese HFE patients and revealed 0.33% carrier rate in the Chinese population. In a Japanese population study, C282Y mutation was not even found in the HFE gene either (19). Our study shows that the rarity of C282Y mutation does not only pertains to Chinese and Japanese but also to Koreans. The rarity of hereditary hemochromatosis in Asians is due to its significant different genotype compared to that of Caucasians. The importance of our finding is that molecular pathogenesis in

many genetic disorders could be quite different in different ethnic backgrounds, necessitating the molecular genetic studies in order to understand the molecular characteristics in their population.

Since we have high incidence of liver disease, determining the prevalence of *HFE* mutation in a group of patients with liver disease could be very important. Bacon et al. (20) demonstrated that 66 patients homozygous for the C282Y mutation of *HFE* had an elevated hepatic iron concentration, but 15% of these patients did not meet a previous diagnostic criteria for hemochromatosis. Determination of *HFE* genotype is clinically useful for Caucasian patients, however, our finding suggests that a diagnosis of hereditary hemochromatosis should be rarely considered in Korean patients with liver disease even with suspected iron overload. Although, the heterozygosity for hereditary hemochromatosis was reported to be associated with more fibrosis in chronic hepatitis C (21), our study excluded the possibility of underlying genetic background which might influence morbidity and mortality in liver disease.

In conclusion, allele frequency of C282Y causing hereditary hemochromatosis in the most Caucasian patients is very rare in the Korean population. Our molecular findings suggest that the rarity of hereditary hemochromatosis in Koreans is due to its significant different genotypes compared to that of Caucasians.

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