RAPID DIAGNOSIS OF HEMOGLOBIN CONSTANT SPRING AND HEMOGLOBIN E BY AMPLIFIED CREATED RESTRICTION SITES

To the Editor:

Hemoglobinopathies are a group of genetic disorders characterized by alteration of the structure of one of the globin chains of the hemoglobin (Hb) molecule. The diagnosis of these disorders depends on Hb electrophoresis, isoelectric focusing, high performance liquid chromatography, and amino acid sequencing. Another approach is genetic analysis. Many hemoglobinopathies are caused by point mutation of globin genes. Traditionally, detection of known point mutations or of small deletions is dependent on allele-specific hybridization, direct sequencing of polymerase chain reaction (PCR) products, ligase-mediated allele detection, or cleavage mismatch detection. If the mutations create or abolish a restriction site, it is usually easy to detect the mutations after digestion of the PCR product by using specific restriction enzymes. Unfortunately, not all of the mutations will create or abolish a restriction site, and some enzymes are very expensive or difficult to handle. To solve these problems, we have devised a nonradioactive method by using site-directed mutagenesis to create specific restriction sites to diagnose two common hemoglobinopathies in Chinese.

In this study, we obtained DNA from 40 individuals with Hb Constant Spring (Hb CS) and from four individuals with Hb E disease.

The diagnoses of these cases were based on cellulose acetate electrophoresis, isoelectric focusing, or direct sequencing of the entire globin genes. For the detection of Hb CS, two pairs of primers were used for PCR. The first pair of primers (upstream primer, 5'-CGTGCTGACCCTCCAGACCCGT-3'; downstream primer, 5'-GTCTGACAGGTAAACACCTCCAT-3') was used to recognize the base “C” of the codon “CAA,” which is the mutant codon at the position of the normal α termination codon. This primer pair can also amplify part of the 3' end of the pseudo a1 gene, but not the a1 gene. The second pair of primers (upstream primer, 5'-AGCCACTGCTGCTGCTGAC-3'; downstream primer, 5'-GAAACGCTACCGAGGCTCAAGC-3') was used to recognize the bases “AA” of the codon “CAA” of Hb CS. The PCR product of the αα gene with the first primer pair will create a restriction site for Thnl and with the second primer pair will create a restriction site for HindIII. Figure 1A shows the results of digestion of PCR products from individuals with and without Hb CS. For αα, a 186-bp fragment was formed after digestion with Thnl and a 122-bp fragment was noted after digestion with HindIII. For normal and other termination codon mutations, only one of the digested fragments could be noted. Case 1 is an Hb H patient with Hb CS with a genotype of αα/α-.. An almost completely digested band (186 bp) was formed after Thnl digestion.
I digestion. The undigested fragment (204 bp) was the amplified 3’
end of the pseudo α1 gene. A completely digested band (122 bp) was
formed after digestion by HindIII. Case 2 is a carrier of Hb CS with
a genotype of αα/ααes. The 186-bp fragment is one-third of the un-
digested PCR product (204 bp). For Hb E disease, the mutation creates
a Mnl I restriction site naturally, but this enzyme is very expensive
for us. To overcome this problem, we introduced a 3-nt mismatch
into the 3’ end of antisense primer (5’-T-GTAACCTTGATACCA-
ACCTGCCCAGAAGCT-3’) to create an AAGCTT restriction site
for HindIII in the mutation case. The normal allele disrupted the
target sequence. The sense primer was 5’-ATCACTTAGACCT-
CACCGCTTGAGCCA-3’. Results of the restriction map alteration
of Hb E disease are shown in Fig 1B. A 277-bp PCR product was
amplified and a novel restriction site of HindIII was introduced into
the PCR products obtained from the mutant allele. After digestion
with HindIII, the restriction fragments of mutant allele (247 bp) were
30 bp shorter than the uncleaved PCR products of the wild-type
allele (277 bp). All the mutants can be distinguished from the wild-
type allele on the basis of fragment size alteration on 3% agarose gel
electrophoresis of the specific restriction enzyme-digested PCR prod-
uct. The DNA amplification was performed as described,3 but we
modified the PCR program. Denaturation at 94°C for 2 minutes,
and extension and annealing at 65°C for 3 minutes were used for
the αα gene. The PCR program for the β0 gene is denaturation at
94°C for 2 minutes, annealing at 50°C for 2 minutes, and extension
at 72°C for 3 minutes. The method used here to detect hemoglobin-
opathies avoids radioisotopes, eliminates complex procedures, and
is much easier to use than other methods. We have found this ap-
proach to be convenient and effective and recommend that it be used
in studying other hemoglobinopathies.

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