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An assay of gene copy number and its application based on heteroduplex products

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ABSTRACT

The aim of this study was to set up a simple and efficient method for detecting gene copy number, based on heteroduplex products from single-tube PCR/DHPLC. Single-nucleotide polymorphisms (SNPs) on the α-globin gene and chromosome 21 were used as examples. And the formula for quantitative calculation of gene copy number was deduced—based on the peak heights of homoduplexes and heteroduplexes on the DHPLC pattern. 27 samples (14 normal DNA and 13 cases of trisomy-21) were assessed with this method, and 160 samples (48 normal DNA and 112 α-thalassemia samples) were assessed with this method combined with a duplex PCR/DHPLC. Results for 184 of 187 cases were concordant with the known genotypes; three cases of trisomy-21 could not be detected because the target SNPs were homozygous. In conclusion, quantitative assessment of heteroduplex products from single-tube PCR/DHPLC is simple and rapid, and can be used to detect α-thalassemia gene deletions ($\alpha^{-3.7}$, $\alpha^{-4.2}$) and trisomy-21.

Introduction

Studies have shown that gene copy number variations (CNVs) are present in many human diseases, such as genetic disorders, autoimmune diseases, and cancer. Scientists are eager to further investigate CNVs because of their potential practical applications for studying genetic diseases in humans (Mileyko et al., 2008; McCarroll, 2008; Norskov et al., 2009). Progress with biotechniques has led to the development of many methods for detecting CNVs, such as PCR and high-throughput arrays. Each method has advantages and disadvantages (Takahashi et al., 2008; Goossens et al., 2009; Kamath et al., 2009). α-Thalassemia, caused by deletion of the α-globin gene, and Down's syndrome (trisomy 21), resulting from an extra copy of chromosome 21, are diseases associated with CNVs. Multiplex gap-PCR has been combined with DHPLC to detect α-globin gene deletions (Hung et al., 2007; Liu et al., 2010), but this method requires multiple DHPLC analyses because of the single-tube aspect of gap-PCR. No studies using DHPLC analysis have reported detection of Down's syndrome. In this study, we quantitatively assessed gene copy number by use of single-tube PCR combined with DHPLC. We assessed DNA samples of three common deletions of α-thalassemia, and of Down's syndrome. The accuracy and suitability of the method were validated.

Materials and methods

Samples

Fifty DNA samples with known genotypes, including −3.7/αα (ten cases), −3.7/− (three cases), −4.2/αα (eight cases), −4.2/− (two cases), αα/αα (14 cases), and trisomy 21 (13 cases), were used to establish and validate the method. One hundred sixty blood samples, including 112 α-thalassemia samples with various genotypes and 48 normal DNA samples, were collected from Liuzhou City, China, from January, 2007 to February, 2010. Twenty-seven blood samples, including 14 normal DNA samples and 13 cases of trisomy 21, were collected from Beijing, China, from March, 2008 to May, 2010. All samples for α-thalassemia testing were submitted to the lab for a blinded study. Informed consent was obtained from all participants. The study conformed to Chinese ethical guidelines for human and animal research and was approved by the Beijing Chaoyang Hospital Ethics Committee.

Primer design for α-globin gene

On the globin gene, sequences α_2 and α_1 are highly homologous, so a universal primer was designed for the 5′ end of α_2 and α_1. The primer sequences are shown in italic in Fig. 1. The PCR product is a 293 base pair (bp) fragment, which is shorter than the 353 bp fragment

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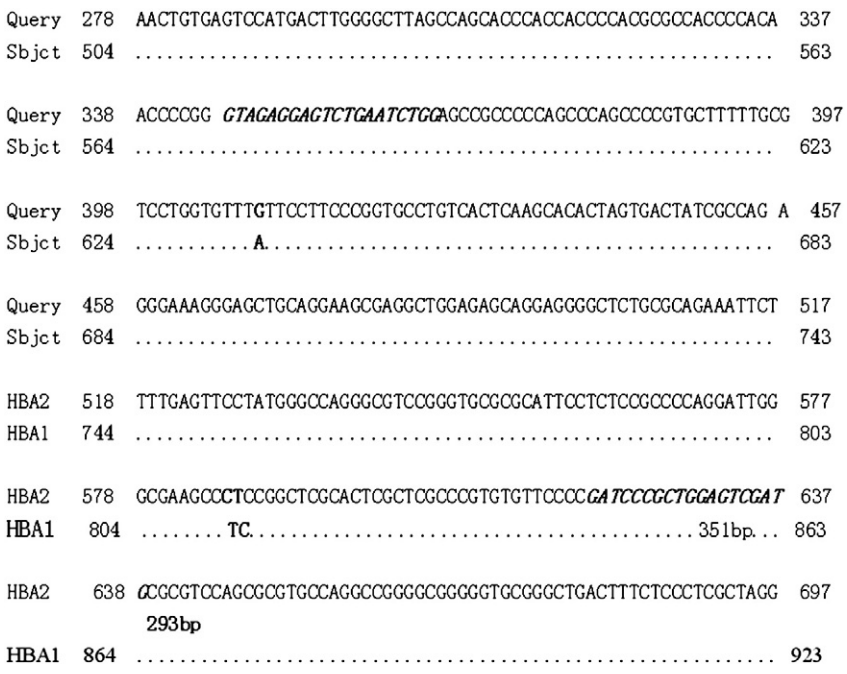

Fig. 1. Primer design for α-globin gene fragment.

reported previously (Hung et al., 2007). There are two differences (3 bps) in the α-globin strands, shown in bold in Fig. 1.

Primer design for chromosome-21 fragment

A sequence fragment (26222060–26222363, 304 bps) with two SNPs on chromosome 21 was designed for amplification, as shown in Fig. 2: primers are in italic and two SNPs are in bold (26222132-C/T, 26222306-C/T).

PCR reaction

The 25 μL PCR mixture contained 2.5 μL 10× buffer, 2.0 μL dNTP (2.5 mmol/L for each), 2.5 μL $MgCl_2$ (2.5 mmol/L), 0.6 μL of each up and down primer (10 μmol/L), 1 μL Gold-Taq DNA polymerase (1 U/μL), and 1 μL of genome DNA (0.1 μg/μL). Negative and positive controls were included. PCR was performed using an ABI Prism 9700 thermocycler (Applied Biosystems, Foster City, CA, USA). PCR conditions were 94 °C for 10 min followed by 40 cycles of 94 °C for 20 s, 61 °C for 30 s, and 72 °C for 40 s. The annealing temperature was decreased by 1 °C each cycle until it reached 59 °C (three cycles), and the final extension was conducted at 72 °C for 5 min. Samples were then used for DHPLC analysis.

DHPLC analysis for gene copy number detection

DHPLC analysis was done on the WAVE nucleic-acid fragment-analysis system (Transgenomic, Omaha, NE, USA) as previously described (Xiao and Oefer, 2001). 5–10 μL of PCR product was analyzed at the melting temperature (application type: mutation detection). After PCR, denaturation at 94 °C for 1 min disintegrated the DNA into four single strands, and they were reintegrated (renatured) by slowly decreasing the temperature. Two new DNA heteroduplexes and two homoduplexes were formed. They were analyzed at melting temperature in a linear acetonitrile gradient with triethylammonium acetate (TEAA) as the mobile phase, using buffer A (0.1 mol/L TEAA) and buffer B (0.1 mol/L TEAA with 25% acetonitrile). The initial buffer concentrations were 51.1% B, with a gradient over the 4.5-min run time to 60.1%, and the flow rate was 0.9 mL/min. The eluted DNA fragments were detected at a wavelength of 260 nm. Heteroduplexes were eluted earlier than homoduplexes when they passed by the DHPLC column. Samples with a single right deletion

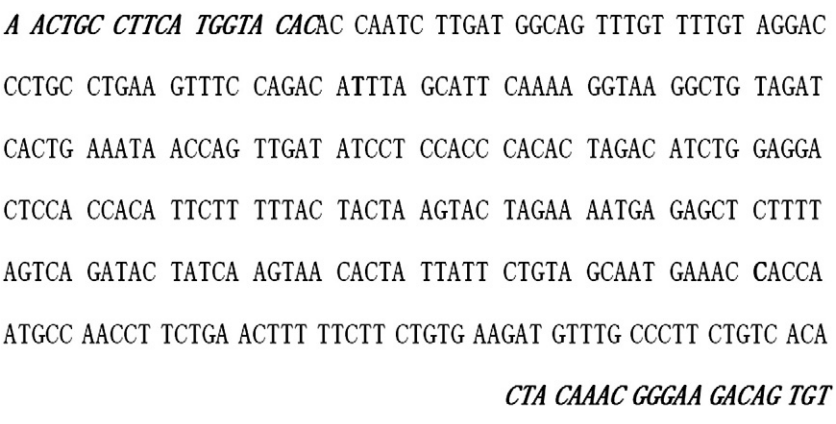

Fig. 2. Primer design for chromosome-21 fragment with SNP sites.