RESEARCH NOTE

DETECTION OF DELETION $\alpha^+\text{-THALASSEMIA MUTATION}$ $[-\alpha\ (3.7),\ -\alpha\ (4.2)]$ BY QUANTITATIVE PCR ASSAY

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Abstract. In Thailand, Hb H ($\alpha^0\text{-thal}/\alpha^+\text{-thal}$) disease is highly prevalent. We designed 3 primer sets (A, B and C) to detect $-\alpha\ (3.7)$ and $-\alpha\ (4.2)$ deletion types of $\alpha^+\text{-thal}$ by quantitative (q)PCR. The A and C primer sets were used to amplify DNA sequences at the 3’ terminal regions of HBA2 and HBA1 gene, respectively, and the B primer set was used to amplify an upstream DNA sequence at the 5’ flanking region of HBA1 gene. The relative quantities of the PCR products (based on threshold cycle (CT) values) of the 3 primer sets were calculated according to the equation $R = 2^{-\Delta\Delta CT}$, and these values were used to distinguish between $-\alpha\ (3.7)$ and $-\alpha\ (4.2)$ deletion mutations. The type of $\alpha^+\text{-thal}$ mutations was determined by calculating the difference between $R_{(C-A)}$ and $R_{(C-B')}$, yielding a value either of 0.5 or 1.0, which indicates the copy number of the target DNA compared with normal diploid control. Measured values that are close to 0.5 indicate there is a single allele of the target DNA. This method was applied to 250 DNA samples recruited for this study, and the $R_{(C-A)}$ and $R_{(C-B')}$ value determined for 185 cases of non-$\alpha$-thal was $1.03 \pm 0.04$ and $0.95 \pm 0.08$, respectively, for 41 cases of $-\alpha\ (3.7)$ $\alpha^+\text{-thal}$ trait $0.49 \pm 0.04$ and $0.45 \pm 0.04$, respectively, and for 2 cases of $-\alpha\ (4.2)$ $\alpha^+\text{-thal}$ trait $0.5 \pm 0.1$ and $1.01 \pm 0.06$, respectively. The allele frequency of $-\alpha\ (3.7)$ and $-\alpha\ (4.2)$ mutation was $0.092$ and $0.004$, respectively. These results were in concordance with those obtained by conventional gap-PCR. The method described here is simple, accurate and feasible for screening of $\alpha^+\text{-thal}$ carriers and should provide valuable information for genetic counselling of patients at risk of having a child with Hb H disease.

Keywords: $\alpha^+\text{-thal}$ deletion mutation, quantitative PCR, relative gene quantification assay