CORRESPONDENCE

The Frequency of α -Globin Gene Triplication in a Southern Chinese Population

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Alpha gene number plays an important role in modulating the phenotype of thalassemia. It has been known that the coexistence of α -globin gene triplication ($\alpha\alpha\alpha$) is an important modulator of the severity of β -thalassemia trait, exacerbating the phenotypic severity of β -thalassemia by causing more globin chain imbalance [1, 2]. Therefore characterization of α -globin gene mutations can be of great help in identification of hemoglobinopathies. Different frequencies of α -globin triplication have already been reported in many populations [3–6]. However, there has been no epidemiological study of α -globin gene triplication in Chinese. Here, we report the prevalence of α -globin triplication in a southern China population with high prevalence of β -thalassemia.

During a one-month period of June 2014, 1169 sequential cord blood samples at birth were collected from the delivery unit, Guangzhou Women and Children Medical Center, Guangdong, China. The samples were frozen and stored at -20°C up to the time of molecular investigation. Genomic DNA was extracted from leukocytes using phenol–chloroform-based method. The multiplex Gap-PCR method described by Wang et al. was applied to screen for the presence of the $\alpha\alpha\alpha^{\text{anti}3.7}$ and $\alpha\alpha\alpha^{\text{anti}4.2}$ triplications [7]. For samples carrying either an $\alpha\alpha\alpha^{\text{anti}3.7}$ or $\alpha\alpha\alpha^{\text{anti}4.2}$ junction, multiplex ligation-dependent probe amplification

(MLPA) was used to confirm the presence of extra copies of α -globin gene. MLPA can also discriminate between heterozygote and homozygote triplication. For samples with either an $\alpha\alpha\alpha^{\text{anti}3.7}$ or $\alpha\alpha\alpha^{\text{anti}4.2}$ junction, the two-round nested PCR strategies were also used to detect the HK $\alpha\alpha$ or anti-HK $\alpha\alpha$ allele according to previous studies [8, 9].

Among the 1169 cord blood samples of newborns with Chinese ethnicity, 11 were positive for $\alpha\alpha\alpha^{\text{anti}3.7}$ junction and 9 positive for $\alpha\alpha\alpha^{\text{anti}4.2}$ junction (Fig. 1). In the 9 samples positive for $\alpha\alpha\alpha^{\text{anti}4.2}$, $-\alpha^{3.7}$ junction was found in 5 cases which were confirmed to carry the HK $\alpha\alpha$ allele. Among the 11 cases positive for $\alpha\alpha\alpha^{\text{anti}3.7}$, no $-\alpha^{4.2}$ junction was detected, thus excluding the presence of anti-HK $\alpha\alpha$ allele. All of the 11 samples with simple $\alpha\alpha\alpha^{\text{anti}3.7}$ triplication and 4 samples with simple $\alpha\alpha\alpha^{\text{anti}4.2}$ triplication were also confirmed to have an extra copy of α -globin gene by MLPA (Fig. 2). Therefore the population-based prevalence of α -globin gene triplication was 0.9 % for $\alpha\alpha\alpha^{\text{anti}3.7}$ and 0.3 % for $\alpha\alpha\alpha^{\text{anti}4.2}$ triplication. The allelic frequencies of $\alpha\alpha\alpha^{\text{anti}3.7}$ and $\alpha\alpha\alpha^{\text{anti}4.2}$ triplications were 0.0045 and 0.0015, respectively.

Triplication is a relatively common numerical change of α -globin gene. The reported frequencies of $\alpha\alpha\alpha^{\text{anti}3.7}$ triplication varied from 0.04 % in Sardinians, 0.36 % in American Blacks, 1 % in a multi ethnic population in The Netherlands to 5 % in Greek Cypriots [3–6]. There was no data available for the prevalence of $\alpha\alpha\alpha^{\text{anti}4.2}$ triplication, which might be due to its rare existence. In this study, we first reported the carrier frequencies of α -globin gene triplications in southern China. Corresponding to the more common of $-\alpha^{3.7}$ than $-\alpha^{4.2}$ deletion, the frequency (0.9 %) of $\alpha\alpha\alpha^{\text{anti}3.7}$ triplication was far higher than that (0.3 %) of $\alpha\alpha\alpha^{\text{anti}4.2}$ triplication. In total, we found a carrier frequency of 1.2 % in southern Chinese individuals for α -globin gene

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Fig. 1 Multiplex Gap-PCR based molecular detection of α -globin gene triplications. Amplification of a 2.5-kb fragment of the *LIS1* gene was included as an internal control. A 1.9-kb fragment was amplified for the anti-3.7 allele, and a 1.7-kb fragment for the anti-4.2 allele

Fig. 2 MLPA analysis of the triplicated α cluster. The y-axis represents the ratio signal as compared to the normal control (ratio 1), on the x-axis the MLPA-probe numbers are ordered chronologically

in 5' to 3' direction along the region. **a** simple $\alpha\alpha^{\text{anti3.7}}$ allele; **b** simple $\alpha\alpha^{\text{anti4.2}}$ allele; **c** simple $\text{HK}\alpha$ allele

triplications. Since β -thalassemia is one of the most common monogenic disorders in southern China, it can be expected that the coexistence of triplicated α -globin gene and β -thalassemia should be a cause of thalassemia intermedia in our locality [10]. Therefore the presence of triplicated α -globin genes should be considered in apparent β -thalassemia carriers who were more anemic and symptomatic than expected. The information on the α -globin gene triplication is also of importance in terms of genetic counseling, especially in families with children affected by β -thalassemia intermedia, while only one parent showed a picture of β -thalassemia on hematological analysis.

We also reported a carrier frequency (0.4 %) of the HK $\alpha\alpha$ allele in our population. The HK $\alpha\alpha$ is an α -globin gene cluster containing both $-\alpha^{3.7}$ and $\alpha\alpha^{\text{anti}4.2}$ junctions [8]. This allele contains an intact $\alpha 2$ gene and a $\alpha 1-\alpha 2$ fusion gene ($-\alpha^{3.7}$) with neither decreased nor enhanced α -globin gene dosage. We have confirmed that compound heterozygosity for HK $\alpha\alpha$ and an in cis deletion of double α genes presents as α -thalassemia trait [11]. It can also be speculated that carriers of β -thalassemia trait are unlikely to suffer any more deteriorative effects when combined with the HK $\alpha\alpha$ allele since its imbalanced α/β ratio is unchanged.

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Compliance with Ethical Standards

Ethical Statement The authors declare that they have no competing interests. The work was carried out according to the principles of the Declaration of Helsinki and approved by the ethics committee of

Guangzhou Women & Children Medical Center. Informed consent was obtained from the parents.

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