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Molecular Diagnostic Tests for α-thalassemia

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Thalassemia is an inherited disorder that affects the production of hemoglobin (Hb) and causes anemia, which can range from insignificant to life threatening. Hemoglobin is the substance in red blood cells that enables them to transport oxygen throughout the body. It is composed of a heme molecule and protein molecules called globins. Adult hemoglobin consists of two identical α-globin chains and two β-globin chains as a tetramer. The α-globin genes (α1 and α2 genes) are cis-linked and are located on each chromosome 16 while β-globin gene on chromosome 11. Abnormalities in α-globin, including base pair mutations in genes and reduction or complete absence of gene expression, lead to imbalance of the α-globin and β-globin chains for production of hemoglobin and then result in α-thalassemia. People of Mediterranean, Middle Eastern, African, and Asian descent are at higher risk of carrying the genes for thalassemia. In Hong Kong, the prevalence of this disease is 4%.

The loss of one (-α) or both (--) of the cis-linked α-globin genes (α1 and α2) are the most common causes of α-thalassemias and is related to a few common genomic deletion, such as $--^{SEA}$, $-(\alpha)^{20.5}$, $--^{MED}$, $--^{FIL}$, $--^{THAI}$, $-\alpha^{3.7}$ and $-\alpha^{4.2}$ on chromosome 16 (Figures 1-2). Patients (--/-α) with hemoglobin H disease develop chronic hemoltytic anemia of variable severity, whereas fetuses (--/--) with Hb Bart's hydrops fetalis die either *in utero* or shortly after birth as a result of severe intrauterine anemia. Individuals with three functional α genes (-α/αα) are clinically and hematologically silent and carriers with α-thalassemia trait (-α/-α or --/αα) only result in very mild hypochromic microcytic anemia. However, couples with these genotypes are at risk of having a hydrops baby or offspring with Hemoglobin H (HbH) disease. In addition to those common deletions, there are two common mutations (TAA ➔ CAA at codon 142 Hemoglobin Constant Spring variant and CTG ➔ CCG at codon 125 Hemoglobin QuongSze variant of α2, Figure 2). These mutations result in reduction of α-globin level, and are frequently identified in Hong Kong Chinese. Patients with the mutation qand the common deletions (--) also develop HbH disease.

Complete blood count is used as an initial screen for thalassemia. The size of red blood cells (mean corpuscular volume, MCV) and hemoglobin amount (mean corpuscular Hb, MCH) as red cell indices are determined and the cutoff values for proceeding to further investigations, such as HbH inclusion test, high-performance liquid chromatography, and genotyping, are MCV <80fL and MCH <27pg. For the HbH inclusion test, peripheral blood is incubated with brilliant cresyl blue and the presence of HbH inclusion bodies suggests α-thalassemia. However, the false positivity rate is very high due to occasional imperfect incubation. Importantly, it cannot differentiate two genotypes of carriers (-α/-α or --/αα). Carriers with --/αα genotype have increased risk for producing Hb Bart's hydrops fetalis, which remains fetal, while no increased risk for -α/-α carriers, who only have risk for producing baby with HbH disease. Usually patients with HbH disease have normal lives and are treatable for late complications. Thus, differentiation of two carriers is important in genetic counseling for those couples and prenatal diagnosis.

In our laboratory, we conduct multiple PCR assays with primers recognized the 5 common deletions ($--^{SEA}$, $-(\alpha)^{20.5}$, $--^{MED}$, $--^{FIL}$, and $--^{THAI}$) (Test 1), $-\alpha^{3.7}$ deletion (Test 2), and $-\alpha^{4.2}$ deletion (Test 3) for these alterations (Figures

1-2). To identify base pair mutation in α-globin genes, DNA sequencing for all the coding regions of both α1 and α2 genes is currently used. The tests are indicated to confirm the diagnosis of α-thalassemia with the context of HPLC or gel electrophoresis data, to differentiate mild-thalassemia from iron deficiency anemia which can be corrected by iron therapy, and to differentiate -α/-α and --/αα carriers in the assessments of risk for producing fetal Hb Bart's hydrops fetalis in genetic counseling.

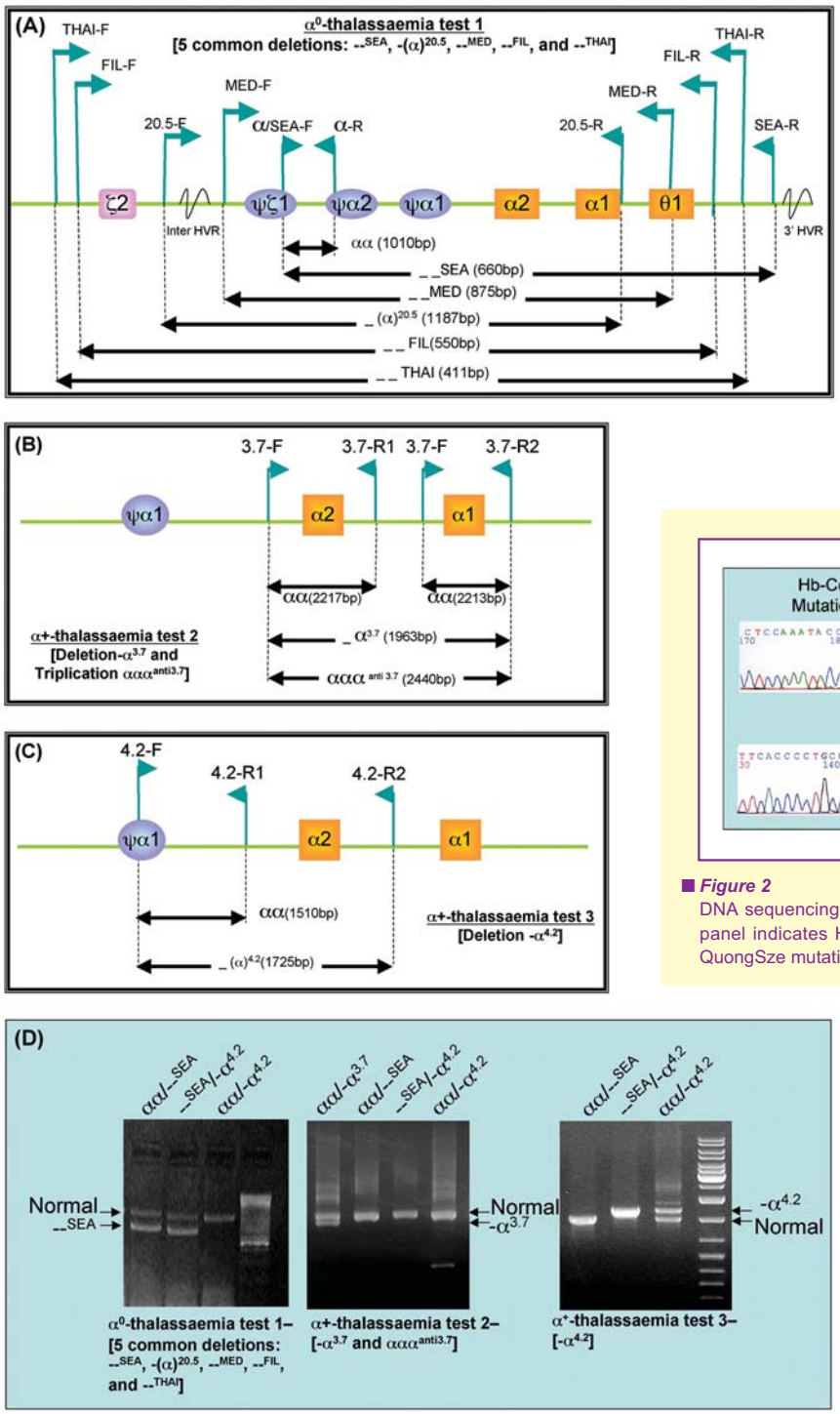

Figure 1

The common α-globin genes deletions in Hong Kong Chinese a-thalassemia patients and the primer designs of multiplex PCR assays for detection of these deletions. **(A)** α⁰-thalassaemia test 1 - Five common deletions [--SEA, -(α)20.5, --MED, --FIL, and --THAI] can be detected by this assay. The PCR product with 1010bp represents the normal α-globin gene cluster and no deletion. The additional PCR products indicate the presence of α-globin gene deletions. **(B)** α+-thalassaemia test 2 – The -α3.7 deletion can be determined by this multiplex PCR and it can also detect the triplication of α-globin genes (ααα anti3.7). The PCR products with 2217bps and 2213bps represent the normal α-globin gene cluster while the presences of 1963 bp PCR product and 2440 bp PCR product indicate -α3.7 deletion and ααα anti3.7 respectively. **(C)** α+-thalassaemia test 3 – The test is used for detection of -α4.2 deletion. The normal PCR product is 1510bp while the PCR product for the presence of -α4.2 deletion is 1725bp. **(D)** The typical gel images of these multiplex PCR assays for detection of common α-globin gene deletion.

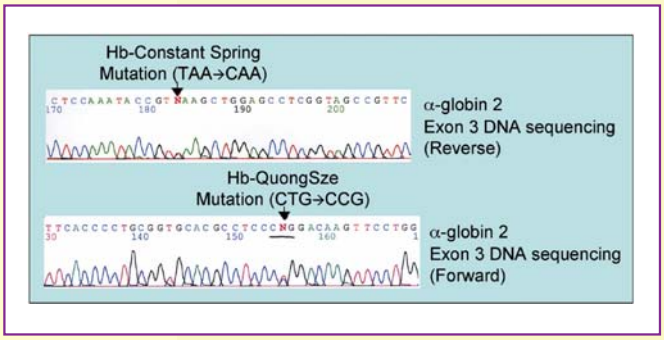

Figure 2

DNA sequencing results of two common mutations in α-globin 2 gene. Upper panel indicates Hb Constant Spring mutation and lower panel indicates Hb QuongSze mutation.

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3. Hardison RC, Chui DHK, Riemer C, Giardine B, Lehvaslaiho H, Wajcman H, and Miller W. Databases of Human Hemoglobin Variants and Other Resources at the Globin Gene Server. http://globin.cse.psu.edu/hbvar/menu.html. Date: 3rd March, 2005.
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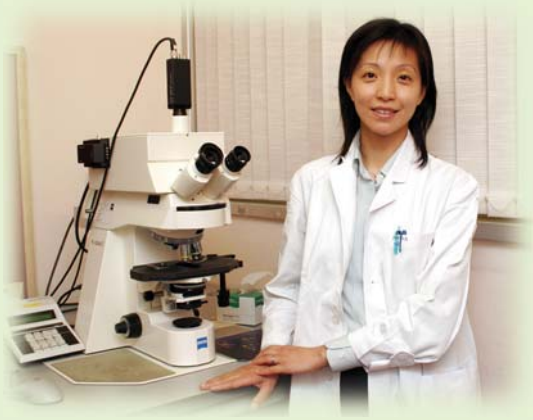
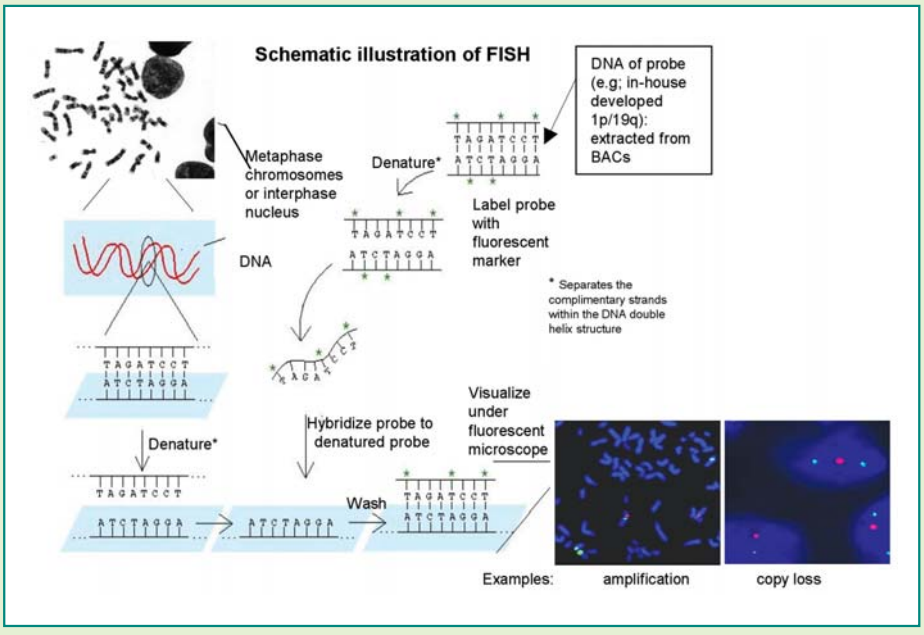
International Oncology Protocol Recommends HER-2/neu FISH Testing for Breast Cancer

Dr. Angela Hui



Molecular cytogenetics are widely applied for management of genomic diseases. One of the mostly frequently used method is Fluorescence-in-situ hybridization (FISH). In FISH, we label probes of interested genes with fluorescent signals. As the target gene or chromosomal region in the interphase nucleus is painted with fluorescent signal, gain or loss of particular gene or genes can be evaluated based on the number of fluoresecent signals detected (see figure). It can also be used to evaluate chromosome number and detect chromosome rearrangements in both cultured cells or paraffin embedded tissues. FISH allows accurate and speedy diagnosis of genetic defects. A further development of the FISH technique is Multi-Color FISH which provides 24 distinct colors to each chromosome. It is very powerful visualization of chromosomal details in a more definitive manner. This colored in marker chromosomes and complex translocations involving undefined chromosomeal partners. We will discuss this technique in the next tissue.

Our group regularly utilizes FISH for detection of 1p/19q deletion in oligodendrogliomas and HER-2/neu amplifications in breast cancers. The HER-2/neu gene is amplified in 25-30% of breast cancers. Amplification of this gene is associated with rapid tumour growth, resistance to chemotherapy and shorter disease-free and overall survival. But these tumours may also respond to anti-HER-2 antibody and also to increased doses of chemotherapy. Assessment of HER-2/neu status is recommended by the American Society of Clinical Oncologists for every breast cancer either at the time of diagnosis or at the time of recurrence. FISH is regarded as an accurate and reliable method for HER-2 assessment.



■ FISH is a technology that utilizing chromosome-specific DNA probes to detect genetic abnormalities that are beyond the resolution of conventional cytogenetics. This is performed by hybridizaing fluorescently labeled DNA probes to the sample DNA (denatured metaphase chromosomes or interphase nuclei). After washing, the signals can be scored under a fluorescent microscope.

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