New streamlined assay can improve prenatal detection of alpha-thalassemia

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In a report in *The Journal of Molecular Diagnostics*, researchers describe a rapid, accurate novel assay for nondeletional alpha-thalassemia genotyping based on one-step nested asymmetric PCR melting curve analysis, which may enhance prenatal diagnosis, newborn screening, and large-scale population screening.

Thalassemia is a group of inherited blood disorders that reduces the ability of blood to circulate oxygen throughout the body. The severity can vary from benign to life threatening; therefore, it is important to identify infants as early as possible who may develop thalassemia-associated symptoms, as well as parents who are carriers. This requires the availability of practical and precise molecular diagnostic tools.

"The nondeletional alpha-thalassemia genotyping assay developed in this study has the advantages of one-step closed-tube operation, high-throughput, speed, and automation, which can meet the methodological needs of a control program for thalassemia in large-scale populations," explained Wanjun Zhou, Ph.D., of the Department of Medical Genetics, School of Basic Medical Sciences, Southern Medical University, Guangzhou, China.

Dr. Zhou noted that the strategy of one-step nested asymmetric PCR melting analysis overcomes the bottlenecks of high homology and GC-rich secondary structure that limited previous types of analyses.

Thalassemia affects up to five percent of the world's population. These

disorders are characterized by low levels of hemoglobin, decreased red cell production, and anemia. Patients with thalassemia report fatigue, weakness, shortness of breath, dizziness, or headaches. One subtype, alpha-thalassemia, is caused by one or more mutations in two different genes (*HBA1* and *HBA2*) associated with production of the alpha-globin subunits of hemoglobin. Every individual has two copies of these genes, so up to four genes can be affected; this can determine the severity of symptoms and carrier status. Though the most common type of genetic mutation associated with alpha-thalassemia is deletional (removal of a section of the gene sequence), the assay in this case focuses on point, or nondeletional, mutations.

The researchers tested the ability of the new assay to detect five nondeletional alpha-thalassemia mutations. All five mutations were accurately identified with a concordance rate of 100 percent in a blind analysis of 255 samples with known genotypes, as determined by other analytic methods including gap- PCR, PCR-reverse dot blot (RDB), or Sanger sequencing.

The investigators also tested the capability of the new assay to screen large populations. After testing 1,250 blood samples, the assay showed 100 percent sensitivity and specificity for all of the targeted mutations.

The overall analysis time with the new assay was just under 2.5 hours. This is considerably faster than other molecular genetic testing methods, such as Sanger sequencing, which require 380 minutes, or RDB, which takes 300 minutes.

"These other methods are unsuitable for use in large-scale screening programs because they have limitations such as cumbersome operation, low throughput, subjective interpretation, and possible laboratory contamination caused by post-PCR open-tube operation," commented Dr. Zhou. "Our results prove that this new assay is accurate, reliable,

simple, and rapid and can meet the requirements for clinical diagnosis and mass screening of nondeletional alpha-thalassemia." He believes the same strategy may be used in the future for rapid genotyping of other genetic mutations.

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