was heterozygous for the αSEA deletion, which removes both the α1 and α2 genes from 1 allele, leaving only 2 functional α genes. This finding explains the comparatively high expression level (40%) of the variant α chain.

In an alignment of 449 α globin sequences from different species, α91 is absolutely conserved as leucine, and this extraordinary level of conservation suggests an important functional role. X-ray structural analyses show that the alkyl side chain heads internally toward the heme plate, where it comes to within 2.4 Å of the Fe2⁺-bound proximal histidine (α87) and within 3.6 Å of the heme (Fig. 1B). Its main chain carbonyl is located on the surface and forms an H-bond with the guanidine group of Arg40 in the adjacent β2 subunit. On oxygenation, movements of the heme Fe2⁺ are transmitted through α87His and α91Leu onto the adjacent β2 subunit to regulate cooperatively and produce the characteristic sigmoidal O₂ binding curve (3). As this model correctly predicts, mutation of β40 Arg also impacts O₂ binding; its mutation to Ser in Hb Austin alters both affinity and cooperativity (4).

There are 2 predictable consequences of the novel α91Leu→Pro mutation, altered O₂ affinity and decreased molecular stability. We were unable to measure O₂ affinity, but we were able to demonstrate decreased molecular stability. A small amount of precipitate was collected after extending a standard isopropanol stability test to 40-min incubation. The precipitate was then dissolved in 1% formic acid and analyzed directly by mass spectrometry (Fig. 1A). This showed selective precipitation of the variant Hb, which was enriched from 40% in lysate to 56% in the precipitate. Only one mutation has been previously reported at this position, α91Leu→Pro. In this case the less conservative mutation was associated with mild anemia and a more marked instability (5).

This case highlights the importance of investigating abnormal Hbs at biochemical, hematological, and genetic levels to understand the pathological implications of the mutation and the risks it poses to carriers.

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References

Stephen O. Brennan1,2
Tim Chan1
Campbell Sheen2

1 Molecular Pathology Laboratory
Canterbury Health Laboratories
Christchurch, New Zealand
2 Pathology Department
Christchurch School of Medicine
University of Otago
Christchurch, New Zealand


To the Editor:
I read with interest the paper by Ho et al. (1), which described a rapid prenatal diagnostic test for simultaneous detection of Hb Barts hydrops fetalis and exclusion of maternal contamination. The main background to their study is that PCR-based techniques are highly sensitive and are also prone to false-positive results in prenatal diagnosis due to amplifica-
tion of contaminating maternal DNA that may be present in the fetal samples. Current established PCR-based diagnostic tests require a separate test to exclude maternal contamination. Hence, the authors developed a novel quantitative fluorescent PCR (QF-PCR) assay that allowed diagnosis and exclusion of maternal contamination in one step. Their approach seemed excellent, with a sensitivity of 100% and specificity of 100%. However, I believe that the presented data did not justify use of the strategy in routine clinical practice.

First, the authors might add details about the incidence of misdiagnosis due to maternal contamination in fetal sampling. They cited a misdiagnosis rate of 3.8% attributable to maternal DNA contamination (2). Actually, this is a high rate of misdiagnosis, unacceptable for an experienced center. We acknowledge that maternal contamination is unavoidable in prenatal sampling, but measures can be taken to make contamination less likely. This problem is usually due to the inexperience of the operator at the time of sampling or of the laboratory technologist engaged in analyzing and separating the fetal material obtained. In our experience, the risk of maternal contamination in chorionic villous samples is remote with careful microscopic dissection to remove contaminating maternal decidua. For amniocentesis, samples contaminated heavily by blood can be cultured for 10–14 days before analysis. Culture of the cells reduces risk of maternal contamination. Although availability of a technique for differentiating tests is important, a low incidence of maternal contamination is vital. A QF-PCR test can easily detect the contamination, but it is no help to diagnosis. When maternal contamination is confirmed, a repeat sampling is usually required, which would add to the risk of contamination (i.e., 3.8%). In our experience in fetal sampling, the misdiagnosis rate due to maternal contamination in all fetal samples is ≤1%.

Second, the QF-PCR approach might not decrease the cost for diagnosis. This technique usually requires both special materials, which increase adds to the cost of regular PCR, and instruments that are not available at most centers. In fact, clinical decision-making for pregnancies at risk for Hb Barts hydrops fetaalis has been changing in regions with a high prevalence of θ-thalassemia. Ultrasonography now plays a major role in early detection of Hb Barts hydrops fetaalis as early as 12–13 weeks, and can effectively differentiate normal pregnancies from those requiring an invasive evaluation (3–5), thus limiting invasive procedures to the few pregnancies identified to be at high risk by ultrasonography. This means that in 3 of 4 at-risk pregnancies, invasive procedures, which carry a 1% chance of miscarriage, are avoided. Because DNA study is relatively expensive, the medical saving might be noticeable. For the few centers with established QF-PCR services for aneuploidy testing, adding this multiplex approach to –SEA diagnosis would be simple and likely to be cost-effective.

Finally, not all prenatal testing requires a test for exclusion of maternal contamination suggested by the authors. For example, we use a regular multiplex Gap-PCR test to detect –SEA mutation of the α-globin gene in fetal samples. If the genotype of the fetus is revealed to be –SEA /–SEA or αα/αα, there is no need to do a differentiating test. If the fetus is revealed to have the same genotype as the mother (–SEA /αα), maternal contamination should be considered. However, due to the high sensitivity and specificity of ultrasonography in detection of Hb Barts hydrops fetaalis, few cases require a repeated sampling.

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References


Dongzhi Li
Prenatal Diagnostic Center
Guangzhou Maternal & Neonatal Hospital
Guangzhou Medical College
Guangzhou, Guangdong 510180
People’s Republic of China
e-mail dongshi3@yahoo.com.cn

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The authors of the article cited above respond:

Diagnosis of a fetal genetic condition requires fetal genetic material, which to date generally implies the need for invasive testing. Ultrasound for evidence of a hyperdynamic circulation can be used as a screening tool to identify at-risk pregnancies, but it is not diagnostic. In time perhaps the use of fetal DNA circulating in maternal plasma may offer more direct evidence of fetal thalassemia status. Many prenatal diagnostic centers now offer quantitative fluorescent PCR routinely for rapid aneuploidy detection. Our test can be readily implemented at only marginal cost. In fact, it eliminates the need for multiple primer sets in several double-deletion α-thalassemia syndromes.

Mahesh Choolani* Sherry Ho Sze Yee1 Samuel Chong2

Departments of 1Obstetrics and Gynaecology and 2Pediatrics
National University of Singapore
Singapore

* Address correspondence to this author at: Department of Obstetrics and Gynaecology, National University of Singapore, 5 Lower Kent Ridge Rd., Singapore 119074; e-mail obgmac@nus.edu.sg.

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