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CASE REPORT

A rare case of compound heterozygous Southeast Asian double α-globin gene deletion and Haemoglobin Quong Sze in a Malay proband

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Abstract

Introduction: Haemoglobin (Hb) Quong Sze is a non-deletional α-thalassaemia subtype that occurs due to missense mutation at codon 125 of the *HBA2* gene. Interaction between Hb QS with Southeast Asian double α-globin gene deletion results in non-deletional HbH disease, which is more severe than deletional HbH. *Case report:* A 3-month-old baby boy was presented with neonatal anaemia and mild hepatomegaly. Full blood count revealed severe hypochromic microcytic anaemia. There was an abundance of HbH inclusion bodies in his red blood cells. High-performance liquid chromatography showed a reduced HbA2 level with the presence of pre-run peak. Capillary electrophoresis showed the presence of HbH and Hb Barts. Molecular analysis found a common α^0-thalassaemia ($--^{SEA}$) in one allele and mutation in codon 125 in the other allele. *Discussion*: Non-deletional HbH disease due to a combination of deletional and non-deletional mutations may present with severe clinical manifestations than those with deletion mutations, which warrants accurate diagnosis using molecular techniques.

Keywords: Haemoglobin Quong Sze, HbH disease, molecular, thalassemia intermedia, α-thalassemia

INTRODUCTION

Haemoglobin (Hb) Quong Sze (QS) is a non-deletional subtype of α-thalassaemia commonly found in Southeast Asia and South China. It is due to the missense mutation at codon 125 of the *HBA2* gene that encodes the α2-globin chain of haemoglobin, which changes the translated amino acid from leucine to proline (CTG → CCG). This causes the α2-globin chain to become extremely unstable and degrade rapidly.[1] The mutation has a stronger effect than the *HBA1* gene that encodes the α1-globin chain.[2] Interaction between Hb QS with Southeast Asian double α-globin gene deletion will result in non-deletional HbH disease, which is usually more severe than deletional HbH disease.[3]

In Malaysia, Hb QS is rare and ethnically, it is seven times more prevalent in Malaysian Chinese compared with Malays.[4] In Indonesia, a rare case of HbH disease has been detected among the people of Chinese descent in that country, which is caused by a compound heterozygous Southeast Asian double α-globin gene deletion ($--^{SEA}$) and Hb QS mutation.[5] A similar variant has also been found in a Thai female patient, who presented with several episodes of haemolytic crisis that necessitated blood transfusion.[3] Here, we describe a similar case, but it is unique in relation to ethnicity as it involves a Malay proband with features of thalassaemia intermedia.

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CASE REPORT

A baby boy was referred to our institution at Day 52 of life for neonatal anaemia. He was delivered full-term via emergency caesarean due to foetal distress, but subsequently achieved good Apgar score and he tolerated breastfeeding orally. Both parents were suspected to be α-thalassaemia carriers. He was born out of a non-consanguineous marriage with two healthy elder sisters aged 4 and 6. Physical examination showed he was pale but not jaundiced, with mild hepatomegaly. Cardiovascular and respiratory examinations were unremarkable.

Full blood count revealed severe hypochromic microcytic anaemia (Table 1). The full blood picture showed anisopoikilocytosis with the presence of polychromatic red blood cell (RBC), nucleated RBC, teardrop cells and target cells. There is an abundance of HbH inclusion bodies in his RBC. High-performance liquid chromatography (HPLC; Variant™; Bio-Rad Laboratories, Hercules, CA) showed reduced HbA and HbA2 levels with the presence of pre-run peak and increased HbF. Capillary electrophoresis (CapillaryS; Sebia, Montpellier, France) showed presence of HbH and Hb Barts. Molecular studies were carried out using multiplex gap-polymerase chain reaction (PCR) for detection of common α-thalassaemia gene deletions [$-^{-3.7}$, $-\alpha^{4.2}$, $--^{SEA}$, $--^{THAI}$, $--^{FIL}$, $--^{MED}$ and $-(\alpha)^{20.5}$]. Multiplex amplification refractory mutation system (ARMS) PCR was performed to detect non-gene deletions [codon 59 mutation or Hb Adana, codon 125 mutation or Hb QS, codon 35 mutation, codon 30 mutation, initiation codon mutation, and termination codon or Hb Constant Spring]. The common α^0-thalassaemia ($--^{SEA}$) was detected in one allele and mutation in codon 125 in the other (Figure 1).

In the ward, the patient tolerated breastfeeding well. He was transfused with packed red blood cells based on weight. He was then discharged and put under paediatric haematology follow-up.

Table 1: Haematological and molecular data of the patient and both parents

	Patient	Father	Mother
Age	3-month-old	36	34
Race	Malay	Malay	Malay
Haematological parameters			
Hb	6.8 g/dL	14.8 g/dL	11.9 g/dL
MCV	71.3 fl	75.9 fl	66.9 fl
MCH	18.7 pg	27.0 pg	21.8 pg
FBP	Hypochromic microcytic RBC with anisopoikilocytosis, polychromasia, nucleated RBC, target cell, teardrop cell and ovalostomatocytosis	Ovalostomatocytosis	Hypochromic microcytic RBC with ovalostomatocytosis and target cell
Hb A	52.7%	96.3%	96.9%
HbA2	0.4%	2.6%	2.6%
Hb F	11.0 %	1.1%	0.5%
Hb H inclusion	Positive	Negative	Negative
Molecular results			
• Deletion (multiplex GAP PCR)	Heterozygous $--^{SEA}$	Not detected	Heterozygous $--^{SEA}$
• Non-deletion (MARM PCR)	Mutation codon 125	Mutation codon 125	
• Genotype	$--^{SEA}/\alpha^{QS}\alpha$	$\alpha\alpha\ /\alpha^{QS}\alpha$	$--^{SEA}/\alpha\alpha$
Serum ferritin	206 ng/mL		

Abbreviations: Hb, haemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; TWC, total white count; Plt, platelet; FBP, full blood count; RBC, red blood cell; HPLC, high-performance liquid chromatography; CE, capillary electrophoresis; PCR, polymerase chain reaction; MARM, multiplex amplification refractory mutation system.

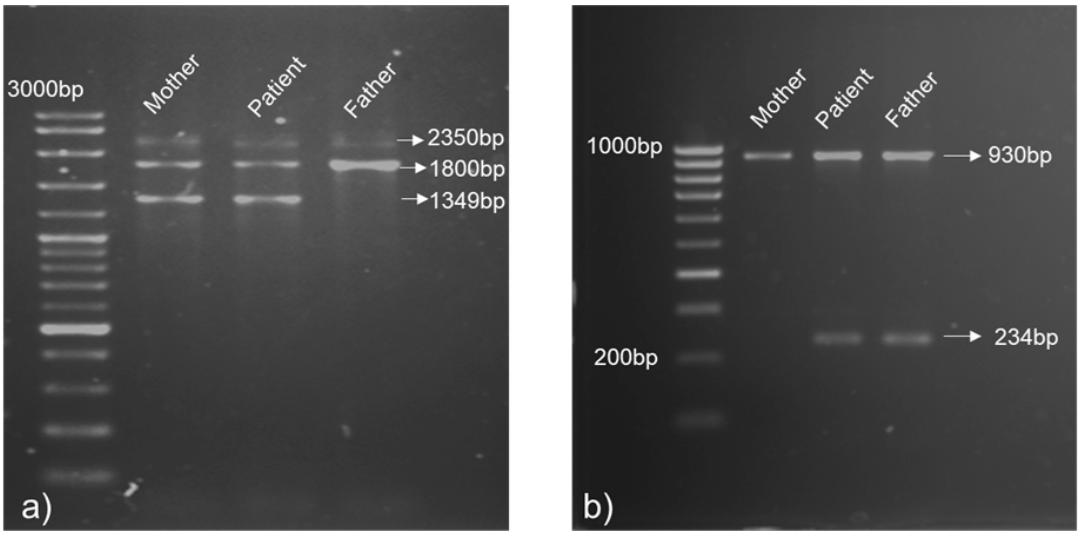

Figure 1: a) Gel electrophoresis image of multiplex GAP-PCR, lane 1: 100bp plus ladder, lane 2 and lane 3: patient's mother and patient carry double gene deletion (--SEA), lane 4: father normal for deletional α mutations. b) Non-deletional gel electrophoresis image. Lane 1: 100bp ladder, lane 2: mother normal for non-deletional α mutations, lane 3-4: patient's father and patient detected to carry codon 125 mutation (Hb Quang Sze)

DISCUSSION

HbH disease is a severe form of α-thalassaemia. The common type is the deletional disease due to three α-globin gene deletions. It is rare to come across non-deletional HbH disease due to a combination of deletional and non-deletional mutations that present with severe clinical manifestations than those with deletion mutations. Detection of HbH-Hb QS is even rarer in the Malay population, making the diagnosis difficult. Hb QS is a very unstable variant, which requires molecular analysis to determine.[6] In a heterozygous state, the individuals are usually asymptomatic, as demonstrated in the patient's father. They may only have mild anaemia with mild microcytosis or normal haemoglobin level. So far, there is no reported case of homozygous Hb QS, most possibly due to under-reporting or rarity of the disease.

The Southeast Asian double α-globin gene deletion ($--^{SEA}$) is one of the common deletional types found mainly in China and Southeast Asia. [3] The majority of α^0-thalassaemia carriers in Malaysia are the Southeast Asian type and it is commonly detected amongst the Chinese.[4] This condition is attributed to the deletion of a nearly 20kb DNA on chromosome 16 that extends from the 3′ end of the *HBZps* gene through the *HbA1* gene (remove Ψα2-, Ψα1-, α2-, α1- and θ1 globin genes).[7] Clinically, the carrier for this genotype will display hypochromic microcytic RBC and reduced synthesis of α-globin chain compared with the β-globin chain. Since this genotype is prevalent in our country, it is easily detected in the patient and mother. However, the clinical features of thalassaemia intermedia warrant further molecular testing for rarer types. In compound heterozygous of Hb QS with Southeast Asian double gene deletion ($--^{SEA}/\alpha^{QS}\alpha$), the patient may present with mild to moderate anaemia, and the symptoms may appear during an infection or pregnancy.[8]

Molecular detection for deletional and non-deletional mutation is important, especially for uncommon variants. In this case, initial detection of the two-gene deletion Southeast Asian genotype still cannot explain the moderate anaemia in this Malay pro-band. Unfortunately, the highly unstable HB QS cannot be detected in routine analysis, therefore the clinician should always consider the possibility of rare genetic mutations before making a final diagnosis. Multiplex GAP-PCR may only detect common gene deletions and to detect non-deletional mutations in α-thalassaemia, multiplex ARMS-PCR is performed.

CONCLUSION

Non-deletion α-thalassaemia causes a severe reduction in α-globin chain production compared

with deletional types. We present a case of a compound heterozygous Hb QS with Southeast Asian double gene deletion (--^{SEA}) in a Malay proband with features of thalassaemia intermedia leading to HbH disease. Even though some cases may only need an intermittent transfusion, others may end up with regular blood transfusion and developmental abnormalities. Therefore, molecular study is essential for an accurate diagnosis to solve the diagnostic pitfalls.

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REFERENCES

1. Qin J, Xu M, Zhang Q, Wen X, He S, Zhou Y, et al. A nested asymmetric PCR melting curve assay for one-step genotyping of Nondeletional α-thalassemia mutations. The Journal of Molecular Diagnostics. 2020;22(6):794-800.
2. Higgs DR. The molecular basis of α-thalassemia. Cold Spring Harbor perspectives in medicine. 2013;3(1):a011718.
3. Sura T, Trachoo O, Viprakasit V, Vathesatogkit P, Tunteeratum A, Busabaratana M, et al. Hemoglobin H disease induced by the common SEA deletion and the rare hemoglobin Quong Sze in a Thai female: longitudinal clinical course, molecular characterization, and development of a PCR/RFLP-based detection method. Annals of hematology. 2007;86:659-63.
4. Ahmad R, Saleem M, Aloysious NS, Yelumalai P, Mohamed N, Hassan S. Distribution of alpha thalassaemia gene variants in diverse ethnic populations in Malaysia: data from the Institute for Medical Research. International journal of molecular sciences. 2013;14(9):18599-614.
5. Widyastiti NS, Nainggolan IM, Kurnia EL, Retnoningrum D, Budiwiyono I. A rare case of Hb H disease caused by compound heterozygous for Î±thalasemia and Hb Quong Sze in Chinese Indonesian proband: a case report. Bali Medical Journal. 2019;8(2):425-8.
6. Al-Madhani A, Pathare A, Al Zadjali S, Al Rawahi M, Al-Nabhani I, Alkindi S. The use of HPLC as a tool for neonatal cord blood screening of haemoglobinopathy: a validation study. Mediterranean Journal of Hematology and Infectious Diseases. 2019;11(1).
7. Farashi S, Harteveld CL. Molecular basis of α-thalassemia. Blood Cells, Molecules, and Diseases. 2018;70:43-53.
8. Li D, Liao C, Li J, Xie X, Huang Y, Wu Q. Hemoglobin H hydrops fetalis syndrome resulting from the association of the—SEA deletion and the αQuong Szeα mutation in a Chinese woman. European journal of haematology. 2005;75(3):259-61.