Hb Q^{India} and its interaction with β–thalassaemia: a study of 64 cases from India

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ABSTRACT

Haemoglobin Q (Hb Q), a relatively uncommon α-chain structural Hb variant, has been reported either in the heterozygous state or interacting with β-thalassaemia. Individuals inheriting Hb Q generally are asymptomatic and are diagnosed by chance during population screening or as a part of a family study. This paper represents the first large study from India of 64 cases of Hb Q, documenting the haematological and molecular findings on 36 cases of Hb Q trait, 22 of Hb Q β-thalassaemia trait and three of Hb Q β-thalassaemia major, as well as a family of Hb Q homozygous cases. Hb Q is detected by Hb electrophoresis and chromatography. Hb Q levels in homozygous cases ranged from 32% to 35%, while in Hb Q heterozygotes the level was 20%. When there was an interaction of β-thalassaemia heterozygotes the level was 14%, and in interacting β-thalassaemia homozygotes the levels range from 7% to 9%. β-thalassaemia mutations were characterised in cases showing elevated Hb A_2 levels, which were markedly reduced in the majority of cases in which β-thalassaemia was absent. Hb Q is rare and not a single homozygous case has been reported. However, Hb Q disease showed wide variation in clinical and haematological presentation in the same family.

KEY WORDS: Hemoglobinopathies.
Hemoglobins.
Thalassemia.

Introduction

Haemoglobin (Hb) Q is a rare α-chain variant first described by Vella *et al*.[1] in association with α-thalassaemia in a Chinese family from Singapore. Structural analysis shows that Hb Q results from the substitution of histidine for aspartic acid in one of the α-chains of Hb A.[2] Three types of Hb Q have been reported in the literature, based on the position of the amino acid replacement, and these are Hb Q^{India} ($\alpha_1 64$),[3] Hb $Q^{Thailand}$ (α74)[4] and Hb Q^{Iran} (α75).[2] Several families of Hb Q interacting with α-thalassaemia have been reported among south-east Asians.

The first case of Hb Q^{India} from India was reported by Sukumaran *et al*.,[3] in 1972 in a Sindhi family with associated β-thalassaemia. Later, a few sporadic cases of Hb Q trait or Hb Q–β-thalassaemia were reported.[5] The present study aims to describe the haematological and molecular findings of Hb Q haemoglobinopathy cases from India.

Materials and Methods

Informed consent was taken from all individuals before blood was collected in EDTA tubes for screening. Red cell indices, examination of red cell morphology, and estimation of fetal Hb levels were performed using standard methods.[6] Red cell osmotic fragility in 0.36% buffered saline was performed using the naked eye single tube red cell osmotic fragility test (NESTROFT).[7]

Haemoglobin analysis was performed using high-performance liquid chromatography (HPLC; Variant haemoglobin testing system, Biorad Laboratories, Hercules, CA, USA) using the β-thalassaemia short programme, and by elution from cellulose acetate membranes after electrophoresis at pH 8.6 for quantitation of Hb A_2 and Hb Q levels. Globin chain electrophoresis on a cellulose acetate membrane (pH 8.9 and 6.0) was carried out to confirm the presence of an α-chain variant.[6]

DNA was isolated from peripheral blood leucocytes using the conventional phenol/chloroform method. Samples with Hb A_2 levels higher than 4% were analysed for β-thalassaemia mutations by covalent reverse dot-blot hybridisation[8] or by the amplification refractory mutation system (ARMS).[9] Presence of the α3.7/leftward deletion was detected by the Bio-Rad mDx Alpha Gene 1 kit in an Hb Q homozygous family.

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Results

A total of 64 cases were identified as showing the presence of Hb Q during screening for haemoglobinopathy over the past two decades. They were picked up initially on cellulose acetate electrophoresis (pH 8.6) in the position of Hb S/D and in association with a double Hb A_2, and were then run on HPLC as described above, eluting at 4.76 min (Fig 1). Globin chain electrophoresis (at acid and alkaline pH) was performed in all the cases and showed a fast-moving α^Q band near the α^A band.

Based on haematological parameters, Hb A_2 levels, molecular analysis for associated β-thalassaemia mutations and family studies, it was possible to divide the cases into

four groups: Hb Q trait (36 cases), Hb Q homozygous (three cases), Hb Q–β-thalassaemia trait (22 cases) and Hb Q–β-thalassaemia major (three cases). The majority (37 cases) originated from Sindh (Pakistan), while the remainder originated from western or northern India, where the patients belonged to the Thacker, Punjabi or Sindhi communities.

Haematological findings in the four groups are shown in Table 1, and the distribution of Hb Q levels is shown in Figure 2. Hb Q levels were below 10% in the majority of cases with interacting β-thalassaemia (7.2–11.3%), while values ranged from 13.1% to 23.5% in Hb Q heterozygotes and from 29% to 36% in Hb Q homozygotes.

The majority were asymptomatic except for the Hb Q homozygous patient who required transfusion support, and the three patients with Hb Q–β-thalassaemia major, who had hepatosplenomegaly and required multiple blood transfusions.

Haematological investigations showed reduced MCV and MCH levels and increased osmotic resistance in all cases of interacting β-thalassaemia. Hb F levels were elevated only in the three multitransfused β-thalassaemia major cases with interacting Hb Q trait. Mild to moderate anaemia was seen in some cases across the four groups (Table 1), but widespread anaemia was not a feature except in the β-thalassaemia major group.

Hb A_2 levels were markedly reduced in the majority of cases in which β-thalassaemia was absent. The β-thalassaemia mutations in the Hb Q–β-thalassaemia trait cases were a 619 bp deletion, FS 8/9 (+G), IVS I–1 (G→T), IVS I–5 (G→C) and CD 30 (G→A). Hb Q–β-thalassaemia major cases had IVS I–1 (G→T) with either 619 bp deletion or IVS I–5 (G→C) mutations.

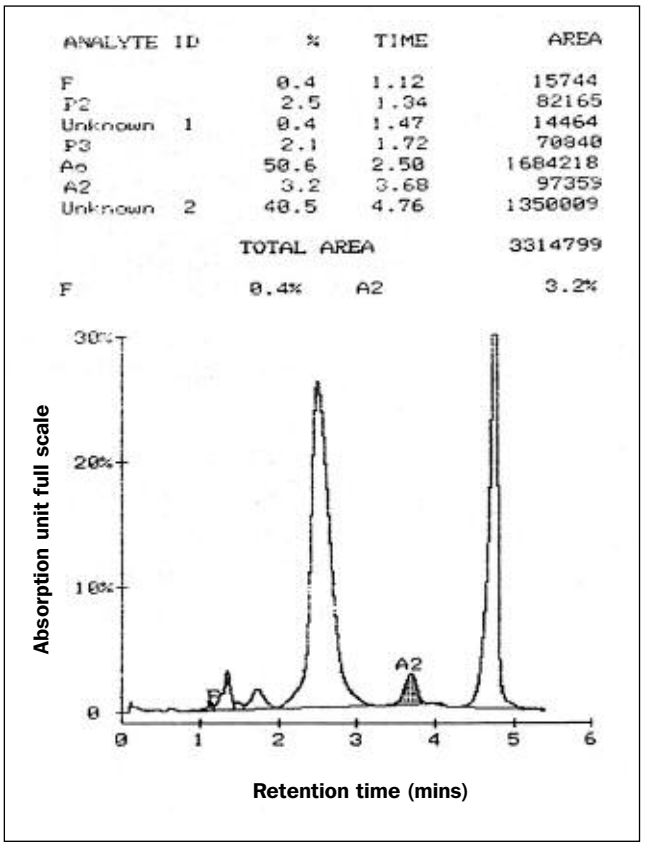

Fig. 1. Chromatogram of Hb Q disease. Unknown 2 peak with a retention time of 4.76 indicates Hb Q.

Discussion

More than 900 Hb variants have been described and almost a quarter are α-chain variants.[10] The Asp→His substitution has also been reported in one γ-chain variant (Hb $F^{Xin\text{-}Su}$) and in five β-chain variants ($Hb^{Karlskoga}$, $Hb^{Summer\ Hill}$, $Hb^{Tigraye}$, $Hb^{Barcelona}$ and Hb^{Yakima}).[10]

Among the α-chain variants reported so far, four substitutions have been reported at codon 64 ($Hb^{Aida/G\text{-}Waimanalo}$, $Hb^{Guangzhou/Hangzhou}$, $Hb^{Persepolis}$ and Hb Q^{India}), while 11 have shown the Asp→His substitution at different positions (Hb^{Sinai}, Hb $L^{Ferrara}$, Hb^{Sealy}, $Hb^{Hasharon}$, Hb $G^{Taichung}$, $Hb^{Mahidol}$, $Hb^{Sunshine\ Seth}$, $Hb^{Sassari}$, Hb $Q^{Thailand}$, Hb Q^{Iran} and Hb Q^{India}.[10]

Joutovsky *et al.*[11] reported six variants (Hb $J^{Toronto}$, Hb $J^{Bangkok}$, Hb^{TyGard}, $Hb^{Twin\ Peaks}$, $Hb^{New\ York}$ and $Hb^{Köln}$) with electrophoretic mobilities identical or similar to Hb S, which could be differentiated and identified by retention time on HPLC. Hb Q^{India}, although showing a similar electrophoretic mobility as Hb S, has a different retention time (4.76 mins) on HPLC.

The first Hb Q variant to be characterised was Hb $Q^{Thailand}$.[2] Hb Q^{India} is an α-globin chain variant that results from a point mutation (AAG→GAG; Asp→His) in codon 64 of the α_1-globin gene on chromosome 16p.[12] The three Hb Q variants do not cause overt haematological disorders because the residue involved is on the surface of the Hb tetramer and the charge changes at these positions do not affect the properties of the Hb molecule.[13] Individuals who are heterozygous for Hb $Q^{Thailand}$ usually show slight red cell microcytosis because the mutation is linked to the $\alpha^{4.2}$ mutation.[14]

As the amino acid replacement is due to a mutation in the α_1-globin gene in Hb Q^{India}, abnormal α^{Q}-chains are synthesised. The α^{Q}-chains combine with the normal β^{A}-chains to form abnormal Hb $\alpha^{Q}_2\beta_2$ (Hb Q). This mutation in the α_1-gene previously was reported in a few north-west Indian individuals belonging to the Punjabi, Sindhi and Lohana communities.[5] Only isolated case reports exist in the literature to indicate its rarity.

Hb Q^{India} has an electrophoretic mobility in the position of Hb S/D at alkaline pH, together with a double Hb A_2 band

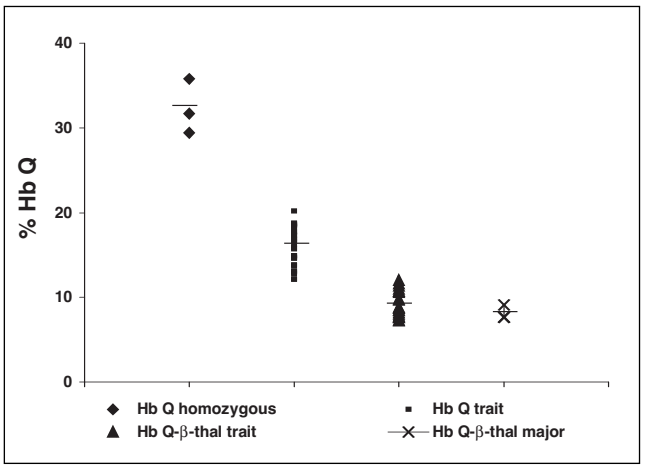

Fig. 2. Hb Q levels in Hb Q haemoglobinopathies.

Table 1. Haematological findings in Hb Q^{India} haemoglobinopathies.

		Homozygous Hb Q (*n*=3)	Hb Q trait (*n*=36)	Hb Q–β–thal trait (*n*=22)	Hb Q–β–thal major (*n*=3)
Hb (g/dL)	<8	1	6	3	2
	8–<10	0	2	5	1
	10–<12	1	9	9	0
	≥12	1	19	5	0
	(Mean±SD)	12.7±1.55	12.55±2.2	11.5±1.4	9.44±1.42
MCV (fL)	≤80	1	19	22	3
	>80	2	17	0	0
	(Mean±SD)	82.8±9.4	78.1±14.4	62.8±6.36	64.8±5.99
MCH (pg)	≤27 pg	1	31	22	3
	>27 pg	2	5	0	0
	(Mean±SD)	28.9±3.4	26.5±5.69	19.6±1.84	21.3±0.43
Hb A_2 (%) $\alpha_2\delta_2$	<2	1	22	0	0
	2–3.5	2	14	0	3
	>3.5	0	0	22	0
	(Mean±SD)	2.4±0.8	1.96±0.53	5.36±0.7	3.3±2.42
Hb Q_2 (%) $\alpha^Q_2\delta_2$	(Mean±SD)	0.73±0.06	0.49±0.16	1.2±0.18	0.4±0.1
Hb F (%)	≤2	3	36	22	0
	>2	0	0	0	3
	(Mean±SD)	0.7±0.3	0.5±0.2	0.8±0.2	12.7±3.29
Hb Q (%)	<10	0	0	18	3
	10–25	0	36	4	0
	>25–40	3	0	0	0
	(Mean±SD)	32.3±3.24	15.7±3.2	9.31±1.9	8.13±0.84

($\alpha^A_2\delta_2$ and $\alpha^Q_2\delta_2$). This minor slow-moving component was thought to represent Hb Q_2. The Hb A_2 levels are reduced in cases without interacting β-thalassaemia, probably as the affinity of α^A-chains for δ-chains is more than that for α^Q-chains. Many times, an abnormal additional Hb Q_2 band is overlooked and this variant is labelled mistakenly as Hb D, due to a negative sickling/solubility test.

Tyagi *et al*.[15] misdiagnosed eight out of 14 cases of Hb Q as Hb D. In the present series, the homozygous Hb Q patient was labelled mistakenly as Hb D trait. If the Hb A_2 variant (here called Hb Q_2) is overlooked, the quantitation of total Hb A_2 will be measured incorrectly, as the total Hb A_2 will be the sum of the 'normal' Hb A_2 and the Hb Q_2.

The abnormal Hb levels for a heterozygous β-chain variant generally range from 35% to 40%, while they are below 25% for heterozygous α-chain variants.[16] Earlier studies have shown that when an α-chain variant and a β-thalassaemia heterozygote interact, the percentage of the α-chain variant ranges from 17% to 30%,[17,18] but when interaction occurs β-thalassaemia homozygotes the levels range from 7% to 9%.[17,19] Results from the present study support these observations.

The three Hb Q homozygotes were from one family but only one case presented with hepatosplenomegaly and portal vein hypertention with oesophageal varices on upper gastrointestinal endoscopy, and required frequent blood transfusions due to the deleterious effect of an increased α^Q-globin chain turnover. There was absence of β-thalassaemia in this family group, which had been report previously.[20]

Hb Q-H disease manifests with chronic anaemia associated with hepatosplenomegaly and jaundice, a thalassaemic blood picture and an absence of Hb A.[14] As the cis form of α-thalassaemia is rare among Indians, no case of Hb Q-H disease has been reported from India. □

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