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Three Novel δ-Chain Variants Identified by DNA Sequencing: δcd 52 [GAT→CAT], δcd 81 [CTC→TTC] and δcd 2 [CAT→AAT]

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ABSTRACT

Background: The mutations in the δ-gene are clinically silent, thus of no clinical significance, yet δ-thalassaemia can mask the presence of ß-thalassaemia trait. A total of 67 mutations have been found in the δ-globin gene of which the majority are structural variants, but the number and different types in the UK population has not been investigated thoroughly at the molecular level.

Patients and Methods: One hundred twenty (120) cases with a suspected HbA_2 variant or δ-thalassaemia were collected for analysis by a genomic sequencing and ARMS-PCR strategy developed for the identification of novel δ-chain variants and δ-thalassaemia mutations. Cases were selected by HPLC analysis showing a shifted HbA_2 peak or a reduced amount of HbA_2.

Results: Twenty eight cases were selected for this publication. Ninty two cases (76.7%) diagnosed as HbA_2' (HbB_2) were published before and excluded from this publication. Ten different variants were identified, including three novel ones. The novel mutations were HBD: c.157G>C, HBD: c.244C>T and HBD: c.7C>A.

The other results were: One case carried HbA_2-Indonesia codon 69 GGT→CGT; 2 cases carried the HbA_2-Coburg mutation, cd 116 (CGC→CAC); 4 patients carried the HbA_2-Babinga, cd 136 (GGT→GAT); 2 cases carried the δ-thalassaemic mutation δ cd 4 (ACT→ATT) $delta^+$; 10 cases carried the HbA_2-Yialousa mutation, cd 27 (GCC→TCC); 1 case carried the Hb A_2-Troodos mutation, cd 116 (CGC→TGC) and 1 case was homozygous for the thalassaemic mutation -68 (C→T). Three of the variants (HbA_2-Indonesia, HBD: c.157G>C and HBD: c.244C>T) had a characteristic shifted retention time on HPLC that can be used for a probable diagnosis.

Conclusions: We are reporting 3 novel δ-globin gene mutations with their genotype/phenotype characterization which can be added to the list of δ-thalassaemia/variants as a database.

INTRODUCTION

At present over 1536 mutations have been found in the human haemoglobin genes the majority in the α- and ß-globin genes. A total of 67 mutations have been found in the δ-globin gene of which the majority are structural variants (HbVar Database). The mutations in the δ-gene are clinically silent as the gene is expressed at low levels, approximately 2-3% of the total haemoglobin **[1]**. Although the disorders of δ-globin are of no clinical significance, δ–thalassaemia can mask the presence of ß-thalassaemia trait **[2]**. A diagnosis of ß-thalassaemia trait is usually based on the presence of a raised HbA_2 (above 3.5%) in combination with reduced red cell indices, the coinheritance of δ-thalassaemia can reduce the HbA_2 level to that found in normal individuals, thus ß-thalassaemia carriers may be missed. Seventeen δ-thalassaemia mutations have been identified to date. δ–thalassaemia mutations can be divided into those which reduce δ-globin synthesis and those in which no δ-globin is produced, designated as δ^+ and δ^0 thalassaemia respectively **[3]**.

The δ-globin structural variants can also complicate the diagnoses of ß-thalassaemia because they may split the HbA_2 peak on HPLC into two equal amounts, and thus may lead to a misdiagnosis for ß-thalassaemia trait if the variant peak is not identifiable, for example due to running with HbA. Thirty two structural variants have been identified in the δ-globin gene, but the number and different types in the UK population has not been investigated thoroughly at the molecular level.

The approach described here applies forward and reverse DNA sequence analysis of the δ-globin gene using a set of primers designed to amplify the whole gene. The method enables both δ-thalassaemia mutations and mutations in the coding sequence that result in δ-chain variants to be identified.

PATIENTS AND METHODS

Blood samples:

Samples were referred to the National Haemoglobinopathy Reference Laboratory in Oxford during a 24-month period in 2007-2009 from the following UK haematology departments for haemoglobinopathy investigations: Walsgrave Hospital, Coventry; St George's Hospital, London; Royal Victoria Infirmary, Newcastle; City Hospital, Nottingham; Royal London Hospital, London; New Cross Hospital, Wolverhampton; Queens Medical Centre, Nottingham; Hope Hospital, Salford and Broomfield Hospital, Essex. All blood samples were sent as whole blood in EDTA for investigation of a possible HbA_2 variant or an abnormally low HbA_2 level. One hundred and twenty cases were identified as carriers of δ-globin gene mutations by cation exchange high performance liquid chromatography (HPLC) analysis. Cases were selected because they had abnormal HbA_2 peak by HPLC, indicating that the patient carried a δ-chain variant or exhibited a reduced HbA_2 level below the normal range or low HbA_2 for *ß*-thalassaemia. In this work, 28 cases out of 120 were DNA sequenced for δ-globin gene mutation; 92 cases out of 120 (76.7%) diagnosed as HbA_2' (HbB_2) were excluded and published before **[4]**.

Haemoglobin analysis:

Full blood counts were performed on all blood samples using the Micros 60 automated cell counter (ABX Diagnostics, Montpellier, France). HbA_2 values were measured by HPLC with the *ß*-thalassaemia short programme on the BioRad Variant-II testing system (BioRad laboratories, Hercules, California, USA). HPLC also identifies δ-chain variants as a separate peak if the variant HbA_2 has a different retention time to normal HbA_2. The HPLC retention time for the normal HbA_2 peak is 3.59-3.68 minutes.

Sequence analysis:

DNA was extracted from whole blood using the standard phenol/chloroform method **[5]**. Amplification of the δ-gene was performed by polymerase chain reaction (PCR) using primers designed to amplify the entire δ-gene (Gene Bank Accession Number: U01317). The forward primer (5' Delta Amp) (5` CAGGGCAAGTTAAGGGAATAGTGG 3`) was designed in the 5` URR sequence and the reverse primer (3' Delta Amp) (5` CAGGCAAAGGAAGGAGGAAGAA 3`) was designed in the 3` URR. The PCR was carried out in a 25 μL reaction containing 12.5 μL Qiagen Mastermix from Qiagen (http://www1.qiagen.com), 2.5 μL Qiagen Q Solution, 1 μL of each primer (10 pmol/μL), 7 μL Sigma molecular grade water from Sigma (www.sigmaaldrich.com) and 1μL genomic DNA. The PCR conditions were 15 minutes at 97°C for the initial step, then 30 cycles of denaturation for 30 seconds at 97°C, annealing for one minute at 65°C, and extension for 2 minute at 72°C then 5 cycles of denaturation for 30 seconds at 97°C, annealing for one minute at 65°C, and extension for 3 minute at 72°C, a final extension period of 10 minutes and a pause at 15°C. PCR amplification was performed in on a Biometra® Uno II machine.

Forward and reverse cycle sequencing was then performed using Beckman CEQ™ DTCS -Quick Start Kit (Beckman, High Wycombe, UK). Three nested forward sequencing primers were designed, one in the 5` UTR (5` GGGCAAGTTAAGGGAAT 3`), one in Intron I (5` ACTGCTGTCAATGCCCTGTG 3`) and one in Intron II (5` ATGCTGATGGGAATAACCTG 3`). Two nested reverse primes were used one in the 3' UTR 5` (ATCTGTAGAGCCTCAGGAAC 3`) and one in Intron II (5` GGAGAAGAGCAGGTAGGT 3`). The products were then processed according to the Beckman Coulter CEQ DNA analysis system user manual (http://www.beckmancoulter. com) and the sequence analysed on a Beckman Coulter CE 8000 automated sequencer according to the manufacturer's instructions.

RESULTS

DNA sequencing of 28 cases of low HbA_2 (other than HbA_2') revealed a δ-globin gene mutation coding for a δ-globin chain variant in 27 patients plus a δ-thalassaemic point mutation in the promoter region in one patient (Tables 1-3). Twenty one (21) cases out of 28 were found to have a δ-globin gene mutation reported previously (http://globin.cse. psu. edu / hbvar/

menu.html) in the Syllabus of Human Haemoglobin Variants **[6]**. Seven cases were found to have novel mutations.

Known mutations:

One case of Asian origin was identified with the δ-variant HbA_2-Indonesia (δ cd 69 GGT→CGT; p.Gly70Arg; HBD: c.208G>C) converting Glycine to Arginine (Fig. 1a). He had normal red cell indices and a split HbA_2 peak totalling 2.7% of the total haemoglobin. The abnormal HbA_2 peak (1.2%) had a retention time of 4.45 minutes (Table 1, Case 1).

Three cases were observed with different mutations at codon 116. Two of them were identified as being heterozygous for HbA_2-Coburg (δ cd 116 CGC→CAC) (p.Arg117His; HBD: c.350G>A) changing the amino acid arginine to histidine (Fig. 1b). These individuals had a low HbA_2 of 1.2% and 1.7% on HPLC. No abnormal HbA_2 variant peak was observed by HPLC (Table 1, Cases 2,3). The third one was identified with the δ-variant HbA_2-Troodos (δ cd 116 (CGC→TGC)) and converts the amino acid arginine to cysteine (p.Arg117Cys; HBD: c.349C>T) and was classified as a δ^+ thalassaemia mutation. No abnormal HbA_2 peak was observed by HPLC. This patient had reduced red cell indices due to iron deficiency, with a low HbA_2 of 1.2% (Table 2, Case 13).

Four cases were found heterozygous for the codon 136 mutation that converts the amino acid from glycine to aspartic acid. This results in the δ-chain variant named HbA_2-Babinga (δ cd 136 (GGT→GAT); p.Gly137Asp; HBD: c.410G>A) (Fig. 1c). None of these cases had an abnormal HbA_2 peak on HPLC analysis (Table 1, Cases 4-7). The mutation for HbA_2-Babinga is not reported to be a thalassaemic mutation and thus normal expression of the variant is expected. The fact that no abnormal peak was observed indicates it is running with one of the other Hb peaks normally observed on HPLC (P2, P3 and Hb A). Three of these four cases were found to have a P3 peak with a slightly shifted retention time of 1.77 minutes instead of the normal P3 retention time of approx 1.65 minutes. One case showed a P3 peak with a shoulder. Thus it is proposed that the HbA_2-Babinga peak is combined with the P3 peak and has a retention time of approx 1.75 minutes. One of these four cases was also a carrier for the ß-chain variant HbS. Individuals with HbS trait have a higher normal range for Hb A_2 (3.5-4.5%) than normal individuals. The case with HbS trait and HbA_2-Babinga had an HbA_2 level of 2.9% (Table 1, Case 6). This correlated with an approximately 50% reduction of the value expected in HbS trait individuals. This case also had a mild reduction in their red cell indices, which was confirmed, by the low % HbS measured by HPLC, to be α^+-thalassaemia trait. The case had 32% HbS, which is in the range of 29-34% observed in individuals with α^+-thalassaemia trait. The other cases with this mutation had normal red cell indices and a reduced level of HbA_2.

Two cases with low HbA_2 (1.7% and 0.6%) were found to have the δ-thalassaemic mutation (δ cd 4 (ACT→ATT) delta$^+$; p.Thr5Ile; HBD: c.14C>T) converting threonine to isoleucine (Fig. 1d). They were also found to be compound heterozygous for δ-thalassaemic mutation (δ cd 27 GCC→TCC; HbA_2-Yialousa) and a silent C→ T at codon 97 (Table 2, Cases 1,2). Both had reduced red cell indices consistent with α-thalassaemia. Gap-PCR revealed that both were homozygous for the common -$\alpha^{3.7}$ mutation. One of them was more anaemic than the other (Hb% were 11.9 and 10.4 respectively) which may account for the too low HbA_2 in the more anaemic one.

Ten cases were found to have codon 27 mutation (GCC→TCC) resulting in the conversion of alanine to serine and has been named HbA_2-Yialousa (p.Ala28Ser; HBD:c.82G>T) and is designated as a δ^+-thalassaemia mutation (Table 2, Cases 3-12) (Fig. 1e). These cases had a low HbA_2 consistent with the δ-gene mutation having a thalassaemic phenotype. Three out of these ten cases were homozygous for the mutation with Hb A_2 between 0.7 and 1% (Table 2, Cases 4,5,7). No normal HbA_2 can be synthesised. The variant HbA_2 peaks had a retention time of 3.62 minutes, which were in the position observed for the normal HbA_2 peak. Thus HbA_2-Yialousa does not have a characteristic retention time. One among these three homozygous also had reduced red cell indices, resulting from α-thalassaemic mutations, as it was shown by DNA analysis (Table 2, Case 7). The other seven cases were heterozygous for the mutation; four among them had also ß-thalassaemic mutations (two were heterozygous for the $ß^0$-thalassaemia mutation IVS1-1 G→A and the other two were heterozygous for the severe $ß^+$-

thalassaemia IVS1-110 G→A); they had reduced red cell indices consistent with this type of thalassaemia which explain their normal HbA_2 (2.8%, 3.3%, 3.1% and 3.4%) (Table 2, Cases 3, 6, 11,12). The other three heterozygous for the mutation, two among them showed HbA_2 of 1.9% and 1.5%; the third one had HbA_2 of 0.6% which was too low for this type of mutation which was confirmed by α sequencing to be due to α-thalassaemia trait (α2 cd 104 TGC→TAC; Hb Sallanches) (Table 2, Cases 8, 9,10).

One case that had low HbA_2 (1.5%) with no abnormal peak on the HPLC profile and normal red cell indices was found homozygous for the δ-thalassaemic mutation (δ -68 C→T; HBD: c.-118C>T) (Table 2, Case 14) (Fig. 1f).

Novel mutations:

Three novel mutations were identified in seven cases. All mutations show characteristic features of δ chain structural variants. The three novel mutations were identified by both forward and reverse sequencing.

One case was a 41-year old male of Indian origin, with reduced red cell indices and a split HbA_2 peak constituting 3.4% of the total haemoglobin. The abnormal HbA_2 peak (1.7%) had a retention time of 4.72 minutes. DNA sequence analysis demonstrated a mutation (HBD: c.157G>C) at codon 52 of the δ-globin gene (GAT→CAT) converting aspartic acid to histidine (Table 3, Case 1) (Fig. 1g).

Three cases had the mutation (p.Leu82Phe; HBD: c.244C>T) at codon 81 of the δ-gene (CTC→TTC), converting leucine to phenylalanine (Table 3, Cases 2,3,4) (Fig. 1h). Among them two cases presented with normal red cell indices and a split HbA_2 peak on HPLC, constituting 2.7% and 2.9% of the total haemoglobin. The abnormal HbA_2 peak (1.3% and 1.5% respectively) had a retention time of 3.82 and 2.9 minutes respectively (Table 3, Case 2,3). The third one showed low HbA_2 (0.5%) and no abnormal peak on the HPLC; it was found heterozygous for the same novel mutation. This case had normal red cell indices and normal α-globin gene screening which could not explain the too low HbA_2 (Table 3, Case 4).

The other three cases with novel mutations had either no or very low HbA_2. DNA sequence analysis demonstrated a mutation (p.His3Asn; HBD: c.7C>A) at codon 2 [δ cd 2 (CAT → AAT)] converting histidine to asparagine (Fig. 1i). One of them was homozygous for the mutation and had no HbA_2 with mild anaemia (Hb: 10 g/dl) with normal red cell indices (Table 3, Case 6). The other two were compound heterozygous for the mutation and the δ^+– thalassaemic mutation (δ cd 27, HbA_2-Yialousa). One of them had no HbA2 with mild anaemia (Hb: 10.6 g/dL) and reduced red cell indices which was diagnosed as iron deficiency [ferritin: 19 µg/L (N: 30-185)] (Table 3, Case 5). The other one had HbA_2 of 0.7% with normal red cell indices (Table 3, Case 7).

Table (1): Known δ-globin gene variants detected in 120 cases of low HbA_2 (other than HBA_2').

Case No	Variant name	HPLC observation	Hb A (%)	δ variant % and rt in min	Hb (g/dl)	MCV (fl)	MCH (pg)	Hb F (%)	Other findings	Ethnic origin
1	HbA_2-Indonesia	Split HbA_2	1.5	1.2 at 4.45	11.8	94	28.9	0.7	–	Far East Asian
2	HbA_2-Coburg	Low A_2	1.2	No abnormal peak	11.5	87	27.9	0.6	–	Dutch
3	HbA_2-Coburg	Low A_2	1.7	No abnormal peak	7.1	66.7	23.3	0.3	–	Cypriot
4	HbA_2-Babinga	Low HbA_2	1.6	No abnormal peak	11.3	93.8	29.7	0	–	Afro-Carribean
5	HbA_2-Babinga	Low HbA_2+ P3 with a shoulder	1.1	No abnormal peak	–	–	–	–	–	Western European
6	HbA_2-Babinga	Low HbA_2	2.9	No abnormal peak	10	–	25	–	32% HbS; α^+ thalassaemia trait	African
7	HbA_2-Babinga	Low HbA_2+ P3 with a shoulder	1.5	No abnormal peak	12.7	99	29.4	1.5	–	African

rt = Retention time.

Table (2): Known δ-globin gene thalassaemic mutations and thalassaemic variants detected in 120 cases of low HbA_2.

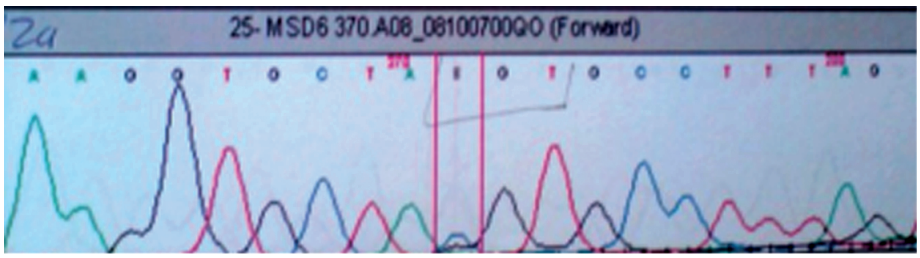
Case No	Variant name	HPLC observation	Hb A_2 (%)	δ variant % and rt in min	Hb (g/dl)	MCV (fl)	MCH (pg)	Hb F (%)	Other findings	Ethnic origin
1	cd 4 (ACT>ATT) delta+	Low HbA_2	1.7	No abnormal peak	11.9	78	22.8	0	$-\alpha^{3.7}$ thalassaemia homozygous; HbA_2 Yialousa;97 polymorphism	Cypriot
2	cd 4 (ACT>ATT) delta+	Low HbA_2	0.6	No abnormal peak	10.4	73	18.3	0.9	$-\alpha^{3.7}$ thalassaemia homozygous; HbA_2 Yialousa; 97 polymorphism	Guyanese
3	HbA_2-Yialousa	Normal HbA_2 for ß-Th	2.8	No abnormal peak		59	19.7		$ß^0$-Thalassaemia trait IVS1-1 G>A by ARMS	Turkish
4	HbA_2-Yialousa	Low HbA_2	0.9	No abnormal peak	11.2	84	28.1	0.6	-	Asian/Malaysian
5	HbA_2-Yialousa	Low HbA_2	1	No abnormal peak	18.1	91	29.3	0.2	-	Egyptian
6	HbA_2-Yialousa	Low HbA_2	3.3	No abnormal peak	10.2	80	23.9	9.1	$ß^0$-Thalassaemia trait IVS1-1 G>A by ARMS	Italian
7	HbA_2-Yialousa	Low HbA_2	0.7	No abnormal peak	11.6	69.7	22.2	0.5	$-\alpha^{3.7}$ thalassaemia homozygous	Pakistani
8	HbA_2-Yialousa	Low HbA_2	1.9	No abnormal peak	13	102	32.8	2.2	-	Polish
9	HbA_2-Yialousa	Low HbA_2	1.5	No abnormal peak	12.3	87	28.3	0.4	-	Iranian
10	HbA_2-Yialousa	Low HbA_2	0.6	No abnormal peak	12.4	78	25.5	0.6	α_2-Thalassaemia cd104 TGC>TAC (Hb Sallanches)	Indian
11	HbA_2-Yialousa	Normal HbA_2 for ß	3.1	No abnormal peak	11.8	64.2	21.5	1.5	B^+-Th (IVSI-110 G>A)	European
12	HbA_2-Yialousa	Normal HbA_2 for ß	3.4	No abnormal peak	12.7	66	21.9	<0.5	B^+-Th (IVSI-110 G>A)	Greek
13	HbA_2 Troodos	Low HbA_2	1.2	No abnormal peak	11.6	72.2	21.3	0	iron def	British
14	- 68 C>T	Low HbA_2	1.5	No abnormal peak	16.3	84	27	1.2		Asian Indian

Table (3): Three novel δ-globin gene mutations.

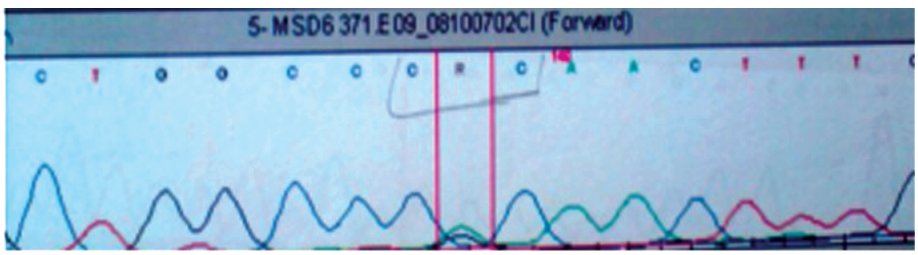
Case No	Variant name*	HPLC observation	Hb A_2 (%)	δ variant % and rt in min	RBC ($x10^{12}$/L)	Hb (g/dl)	MCV (fl)	MCH (pg)	Hb F (%)	Other findings	Ethnic origin
1	HBD:c. 157G>C	Split HbA_2	1.7	1.7 at 4.72	5.02	12	74	23.9	0.3	–	Indian
2	HBD:c. 244C>T	Split HbA_2	1.4	1.3 at 3.82	3.98	12.5	98	31.5	0.5	–	South Korean
3	HBD:c. 244C>T	Split HbA_2	1.4	1.5 at 2.9	4.46	13.7	95	30.6	0.9	–	Japanese
4	HBD:c. 244C>T	Low HbA_2	0.5	No abnormal peak	3.78	12.2	94	32.3	0	Normal α-globin gene screen	Irish
5	HBD:c. 7C>A	No HbA_2	-	No abnormal peak	4.44	10.6	83	23.9	1.6	Ferritin=19 (N: 30-85 ug/L). HbA_2 Yialousa	Asian Indian
6	HBD:c. 7C>A	No HbA_2	-	No abnormal peak	3.53	10	87	28.4	0.6	–	Unknown
7	HBD:c. 7C>A	Low HbA_2	0.7	No abnormal peak	4.01	12.7	100	31.7	3.4	HbA_2 Yialousa	Pakistani/ Indian

* HGVS nomenclature.

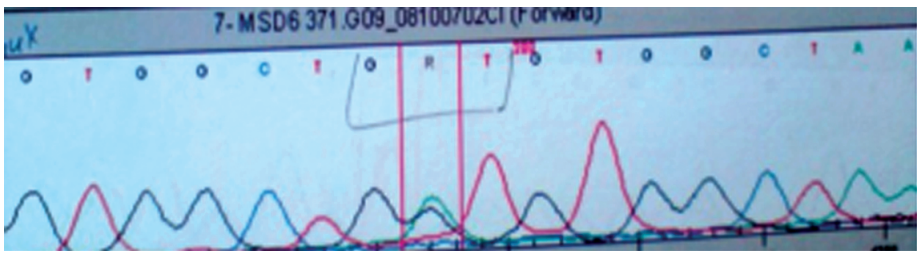
Fig. (1): DNA sequencing chromatograms of the mutation detected (except HbA_2 Troodos) show the depression of the normal wave by the half and the presence of the abnormal wave. f is homozygous, so there is complete replacement of the wave.



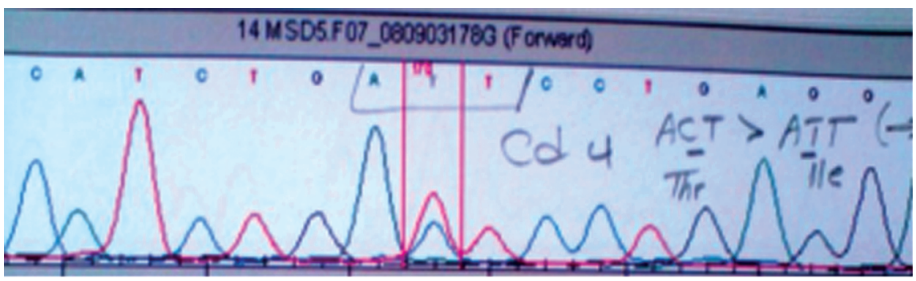
(A): Cd 69 mutation (GGT>CGT), Hb A2 indonesia (HBD:c.208G>C).



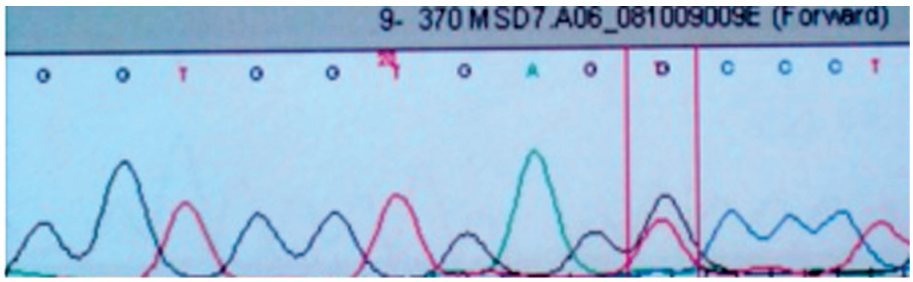
(B): Cd 116 mutation (CGC>CAC), Hb A2-Coburg (HBD:c.350G>A).



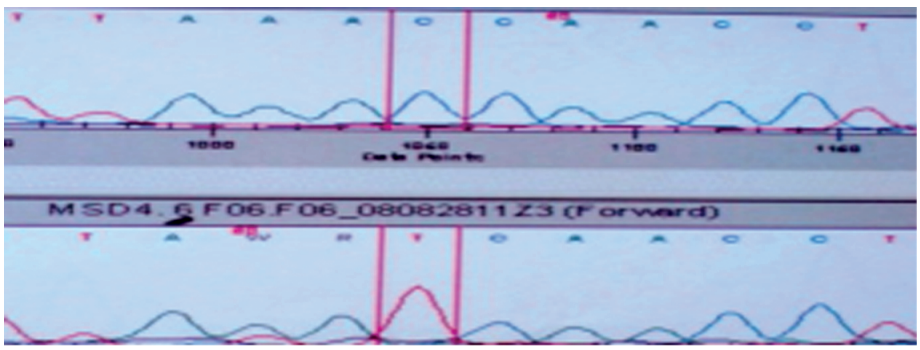
(C): Cd 136 mutation (GGT>GAT) Hb A2–Babinga (HBD:c.410G>A).



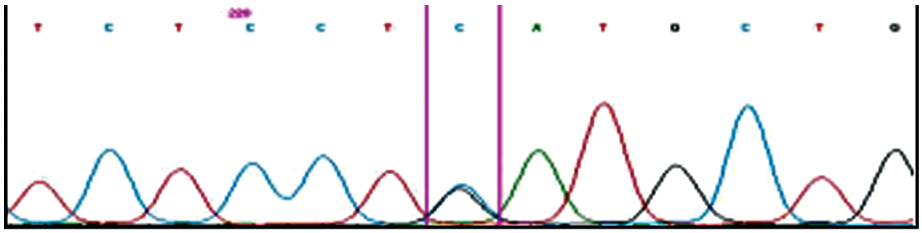
(D): Cd 4 mutation (ACT>ATT) (HBD:c.14C >T).



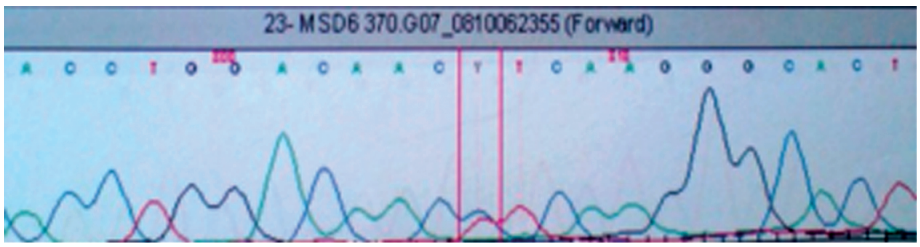
(E): Cd 27 mutation (GCC>TCC) Hb A2–Yialousa (HBD: c.82G>T).



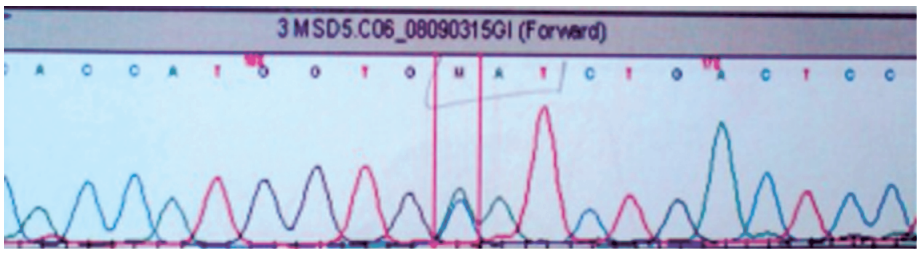
(F): -68 mutation (C> T) (HBD c.-118C>T).



(G): Novel cd 52 mutation (GAT-CAT) (HBD: c.157G>C).



(H): Novel cd 81 mutation (CTC>TTC) (HBD: c.244C>T).



(I): Novel cd 2 mutation (CAT>AAT) (HBD: c.7C >A).

DISCUSSION

In this study 120 case of low HbA_2 were examined by DNA analysis; 92 cases out of 120 (76.7%) diagnosed as HbA_2' (HbB2) were excluded and published before **[4]**. Twenty eight cases out of 120 were DNA sequenced for δ-globin gene mutation.

One case out of 120 (0.8%) of Far East Asian origin was identified with the δ-variant HbA_2-Indonesia. It had been described in the Indonesian and Malay population at low frequency and in association with HbE and with ß–thalassaemia **[7,8]**. This case had normal red cell indices and a split HbA_2 peak constituting 2.7% of the total haemoglobin. The abnormal HbA_2

peak (1.2%) had a retention time of 4.45 minutes which can be useful in identification on the HPLC.

Two cases, from Holland and Cyprus, out of 120 (1.7%) have been identified as being a carrier for variant HbA_2-Coburg **[6]**. This mutation has been previously described in a Sicilian family in association with ß-thalassaemia trait **[9]**. Hb A_2 is low at 1.2-1.7% and there was no identifiable abnormal HbA_2 peak. HbA_2 Coburg is a δ-chain variant without any reported reduction in expression and thus does not appear to have a thalassaemia phenotype. This means that the variant δ-chain peak representing HbA_2-Coburg may run in a hidden position on the HPLC i.e. masked by HbA, P2 or P3 peaks, thus this variant is not identifiable by a characteristic HPLC retention time.

The codon 136 mutation found in four cases out of 120 (3.3%) produces the δ-chain variant Hb A_2-Babinga, previously reported in Babinga Pygmies (1-2%) and a small number of Black families in the United States **[10]**. Two of the four cases were known to be of African origin, one of these being a carrier for HbS, which is commonly found in African communities. Our studies show that Hb A_2-Babinga does not have a characteristic retention time that can be used for its identification by HPLC. Our HPLC results suggest it runs with the P3 peak on HPLC, causing a slight shift in retention time of the P3 peak, which could possibly be of use in identifying the presence of this variant.

Fourteen of the cases were found to have mutations known to cause δ^+-thalassaemia trait. Two cases (Cypriot and Guyanese) out of 120 (1.7%) were found to have the δ^+-thalassaemic mutation (Codon 4 (ACT->ATT) $delta^+$; HBD: c.14C>T). They were also found to be compound heterozygous for Hb A_2-Yialousa and a silent C>T at codon 97. This thalassaemic variant had been described in Greek Cypriots associated with a C>T at - 199, a silent C>T at codon 97, and an AT deletion at position 722 in IVS-II **[11]**. Both had reduced red cell indices and were found homozygous for the common $-\alpha^{3.7}$ mutation.

Ten cases out of 120 (8.3%) had the δ-globin gene mutation HbA_2-Yialousa indicating that HbA_2-Yialousa is the common δ-thalassaemic mutation and the second common HbA_2 mutation in general. HbA_2-Yialousa has been previously described in a Cypriot family **[12]**, however our cases were of different ethnic origin which means it could be ubiquitous. Three cases were homozygous for this mutation and consequently had an extremely low HbA_2 peak of 0.7-1%, which ran in the normal HbA_2 position. Homozygosity for this mutation means that the variant must have the same retention time as HbA_2 and thus cannot be identified by a characteristic HPLC retention time. Four cases were found compound heterozygous for the mutation and ß-thalassaemia mutations, suggesting that HbA_2-Yialousa is the common δ-thalassaemic mutation associated with ß-thalassaemia in our population. Their HbA_2 level were within normal and low for ß-thalassaemia (2.8-3.4%), indicating that HbA_2-Yialousa could compromise the diagnosis of ß-thalassaemia heterozygosity when this is based on the HbA_2 level only.

One British case out of 120 (0.8%) had the δ-thalassaemic variant HbA_2-Troodos previously described in a Cypriot family **[12]**. No abnormal HbA_2 peak was observed for this patient, but it is not clear if the reduced amount of variant HbA_2 runs with normal HbA_2 or is masked by other Hb peaks.

One case of Asian Indian origin out of 120 (0.8%) had the δ-thalassaemic mutation δ-68 C→T (HBD: c.-118C→T). This mutation had been described by Bouva et al. **[13]**, and was localized on the AACCAAC sequence [HBD from c.-120 to -114 (δ -70 to -64)]. The sequence is considered to be a regulatory element and can compromise the diagnosis of ß-thalassaemia carrier.

One case out of 120 (0.8%) with a novel mutation at codon 52 (HBD: c.157G>C) was observed in a patient of Indian origin with a split HbA_2 on HPLC. The variant peak expression (1.7%) was the same as the normal HbA_2 peak expression (1.7%) and thus the variant mutation does not have a thalassaemic phenotype. The new variant has a characteristic retention time of 4.72 minutes which can be used for identification by HPLC.

A second novel mutation was discovered in three cases (South Korean, Japanese and Irich) out of 120 (2.5%). The mutation, at codon 81

(HBD: c.244C>T), also appears to be a δ-chain variant without a thalassaemia phenotype as the HPLC profiles indicate the presence of a split HbA_2 with peaks of equal size. The new variant has a characteristic retention time of 2.9-3.82 minutes, which can be used for identification by HPLC. One case among these three could not be explained for its result (Table 3, Case 4).

The third novel mutation was at codon 2 (HBD: c.7C>A). Three cases (Asian Indian and Pakistani) out of 120 (2.5%) were found to have the mutation. One of them was homozygous for the mutation with no HbA_2 on the HPLC which means that it is δ^0-thalassaemia mutation. The other two were compound heterozygous for the mutation and the common δ^+-thalassaemic mutation (HbA_2-Yialousa); one of them had no HbA_2 which can be explained by mutations in-trans i.e. the two δ-genes are mutated. The other one had Hb-A_2 of 0.7% which can be explained by mutations in-cis i.e. one δ-gene has the two mutations leaving one δ-gene intact.

Although our data do not reflect the real epidemiology of δ-molecular defects in the UK due to the selection criteria used for detection, they do suggest that δ-globin gene defects are very common, raising the possibility of misleading the diagnosis of ß-thalassaemia carriers. In cases with HbA_2 variants, it is important to consider the HPLC chromatogram carefully to avoid an incorrect diagnosis concerning ß-thalassaemia.

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