Known and new δ globin gene mutations and their diagnostic significance

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Mutations in the δ-globin gene (*HBD*, MIM# 142000) are not pathologically relevant. However, since high HbA_2 levels are diagnostic for β-thalassemia trait and a lowered level for an α- or δ-mutation, co-inheritance of δ- and β-gene defects may lead to misinterpretation of diagnostic results. We examined 29 cases with low HbA_2 level diagnosed in our laboratory, in the presence or absence of a second HbA_2 fraction. We found a δ globin gene mutation in 20 cases.

In total four different known mutations were found, three structural and one expressional. Moreover, two new defects were observed, one causing a structural abnormality and one a δ-thalassemia.

The structural abnormality *HBD* c.431A→G (p.His144Arg)(δcd 143 CAC→CGC) was homologous to the β-globin gene variant called Hb-Abruzzo and we have named this mutation HbA_2-Abruzzo. The new δ-thalassemia defect *HBD* c.-118C→T (δ -68 C→T) has no homology on the β-globin gene (*HBB*, MIM# 141900). All mutations caused a low HbA_2 level and through this could lead to misdiagnosis when inherited together with a β-thalassemia.

Key words: HDB, HbA_2, δ-thalassemia, δ-variant.

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In normal individuals over 2 years old the HbA_2 fraction accounts for between 2.5% and 3.4% of the total hemoglobin. Although some silent β-thalassemia traits do not present with elevated HbA_2 fractions, the finding of a slightly to clearly elevated HbA_2 fraction (3.5-8%) is the classic parameter associated with β-thalassemia trait. Conversely, low-normal HbA_2 levels may be associated with α-thalassemia and iron deficiency.[1] Since HbA_2 consists of two α and two δ polypeptide chains, defects on the δ-gene (*HBD*, MIM# 142000) may also modify the expression of the HbA_2 fraction. Co-inheritance of δ- and β-thalassemia results in a less elevated HbA_2 level and may therefore lead to a wrong diagnostic conclusion. Cases in which a δ-gene mutation is present are often observed during diagnostic investigations in our reference laboratory. Here we report the suspected cases of δ globin gene mutations observed during about 2 years of diagnostic activity.

Materials and Methods

We examined a total of 29 cases of which 13 were suspected to have expression defects and 16 to have structural abnormalities. Analytical methods included alkaline starch gel electrophoresis and automatic high performance liquid chromatography (HPLC) analysis (Figure 1). DNA was either extracted automatically using the Autopure LS extractor (Gentra System, Minneapolis, Minnesota, USA) or isolated by selective lysis[2] and high salt extraction.[3] Polymerase chain reactions (PCR) were carried out in the GeneAmp PCR system 9700 (Applied Biosystems, Perkin Elmer Corporation, Foster City/Ca, USA), using three different primer sets. Primer set 1 (5'agaacagccaatctcagggc3'/5'gagcctctcttataaccttg3') gives a fragment of 330 bp. The primers for exon 2 (5'caaggttataagagaggctc3'/5'agagaaaagtgaagcatctc3') overlap the end of fragment 1 and give a 373 bp fragment. The last primer set (5'tgtaaaacgacggccagtgttaacatatgcatgtatctgcc3'/5'caggaaacagctatgaccgaaattaatcaggaagttgagctg3') gives a 347 bp fragment including exon 3. The PCR were conducted with an annealing temperature of 65°C for 1 min and 35 cycles. The DNA was sequenced using an ABI Prism 3730 DNA sequencer (Applied Biosystems, Perkin Elmer Corporation, Foster City, CA, USA).

Results and Discussion

Out of the 29 suspected cases, 20 were confirmed to have either a known or a new point mutation defect.

Known mutations

$HbA'2$ or HbB_2. Ten patients were found to be carrying the common HbB_2 mutation [*HBD* c.49G→C (p.Gly17Arg) (δcd 16 GGC→CGC)][4] (Figure 1C). In two of these cases with low HbA_2 levels (1.6%), heterozygosity for $-\alpha^{3.7}$ α^+-thalassemia was found. In one case with HbA_2 = 1.5%, the same α-deletion was present in the homozygous state. In another, heterozy-

gosity for the HbC [*HBB* c.19G→A (p.Glu7Lys)] was present (HbC; 38.7%, HbA_2; 1.8%).

HbA_2-Yialousa. The δ globin gene mutation HbA_2-Yialousa [*HBD* c.82G→T (p.Ala28Ser)(δcd 27 GCC→TCC)],[5] was found in four independent patients. In one the HbA_2 level was 1.7% and heterozygosity for $-\alpha^{3.7}$ α^+-thalassemia was present. Another patient (HbA_2=1.6%) was heterozygous for $--^{Med\ I}$ α°-thalassemia.

HbA_2-Etolia. One patient with an HbA_2 level of 1.4% had the rare mutation called HbA_2-Etolia [*HBD* c.257T→C (p.Phe86Ser)(δcd 85 TTT→TCT)].[6]

HBD c.275dupT. The frame shift *HBD* c.275dupT (p.Leu92fs)(δcd 91 +T)[7] was detected in one patient with an HbA_2 level of 1.5% and an elevated HbF fraction (2.3%). This patient also had a new mutation on the ^{G}-γ globin gene (*HBG2*, MIM# 142250), c.-90A→T (γ -37 A→T).[8] These data are summarized in Table 1.

New mutations

HbA_2-Abruzzo. In three patients from a single family (father and two children), all carriers of sickle cell trait [*HBB* c.20A→T] with normal levels of HbS (41.9%; 40.4% and 41.4% respectively), a new δ globin gene mutation was observed at *HBD* c.431A→G (p.His144Arg)(δcd 143 CAC→CGC). This mutation, new on the δ gene, is described at the same position on the β-globin gene as Hb-Abruzzo[9] and was named HbA_2-Abruzzo.

HBD c.-118C→T. A new δ-thalassemia was found in a patient with a low HbA_2 level (2.0%). The mutation (*HBD* c.-118C→T)(δ -68 C→T) is localized on the AACCAAC sequence [*HBD* from c.-120 to -114 (δ -70 to -64)], where another mutation, also causing δ-thalassemia, was described by Papadakis *et al*. in 1997.[10] These data are summarized in Table 1.

In nine out of 29 cases no association between the low HbA_2 levels and δ-globin genes mutations was found. Further investigation revealed iron deficiency in three out of these nine cases. A low mean cell hemoglobin as a consequence of iron deficiency may cause a fractional decrease of the HbA_2 level due to an artifact related to the lower amount of total hemoglobin loaded on the HPLC column.[1] In the remaining six cases particular integration conditions of the HPLC diagram could explain the lower estimation (*data not shown*). The mutations found in the 20 remaining cases are discussed below.

HbA'_2 or HbB_2. In ten out of 20 samples HbA'_2 (Figure 2A) was found in a heterozygous form. This polymorphism, inducing the substitution of the amino acid glycine with an arginine, also called HbB_2, is the most common δ chain variant, frequently found in Africans. The $-\alpha^{3.7}$ α^+-thalassemia rightward deletion was found in three of these ten cases (one homozygous, two heterozygous). The rightward deletion in the homozygous state did reduce the total HbA_2 level (HbA_2 +HbB_2) to 2.2%, while the rightward deletion in the heterozygous state apparently did not (2.9%). One patient was a carrier of the HbC variant and had an HbA_2 level of 1.8%, which is significantly lower than in normal carriers of HbC. Due to the division of the HbA_2 into two fractions in the presence of HbB_2 the automatic estimation of the HbA_2 fraction is always substantially reduced (≈1.65%). One should always be alert to additional HbA_2 peaks (Figure 1C) when the diagnosis of β-thalassemia trait is based only on the estimation of the HbA_2 fraction.

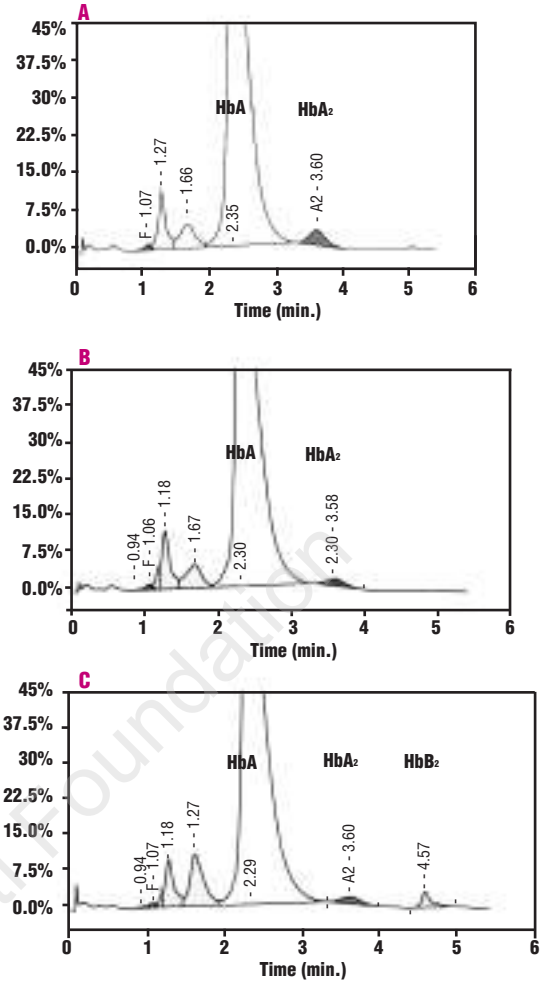

Figure 1. Results of high performance liquid chromatography. **A.** HPLC results of a normal sample. **B.** HPLC results of the sample with HbA_2-Etolia. There is less HbA_2 and no variant is visible. This supports the assumption that HbA_2-Etolia is unstable. **C.** HPLC results of a sample with HbB_2. Besides a lowered HbA_2 level, the variant is also visible.

HbA_2-Yialousa. In the three patients found to be heterozygous for the HbA_2-Yialousa mutation, no abnormal HbA_2 fraction was observed. This variant is caused by an alanine to serine substitution as a result of a G→T transversion at c.82 (δcd 27)(Figure 2B) and results in a δ^+-thalassemia phenotype.[5] Besides having the δ-globin gene mutation, one of the patients was heterozygous for $-\alpha^{3.7}$ α^+-thalassemia deletion and another one for $--^{Med\text{-}I}$ α°-thalassemia deletion. HbA_2-Yialousa could compromise the diagnosis of

Table 1. Patients' data.

Sample no.	Suspected δ gene defect	HbA_2%	HbF %	% HbA_2/X on HPLC	Electrophoresis	δ gene mutation* (HUGO nomenclature)	Other mutations (HUGO nomenclature)	Ethnic origin
1	Hb variant	2.2	0.8	2.5 HbS-like	Slow HbX	HBD c.49G→C		West African
2	Hb variant	1.5	0.7	0.8 HbS-like	Slow HbX	HBD c.49G→C	$-\alpha^{3.7}/-\alpha^{3.7}$	Surinam
3	Hb variant	1.9	0.3	1.6 HbS-like	Slow HbX	HBD c.49G→C		Surinam
4	Hb variant	1.4	0.3	1.5 HbS-like	Slow HbX	HBD c.49G→C		West African
5	Hb variant	1.8	0.6	1.5 HbS-like	Slow HbX	HBD c.49G→C	HBB c.19G→A	Surinam
6	Hb variant	1.3	0.5	1.3 HbS-like	Slow HbX	HBD c.49G→C		Morocco
7	Hb variant	1.5	0.7	1.2 HbS-like	Slow HbX	HBD c.49G→C		Surinam
8	Hb variant	1.6	0.2	1.3 HbS-like	Slow HbX	HBD c.49G→C	$-\alpha^{3.7}$	Surinam
9	Hb variant	1.6	0.2	1.5 HbS-like	Slow HbX	HBD c.49G→C	$-\alpha^{3.7}$	West African
10	Hb variant	1.7	1.6	1.5 HbS-like	Slow HbX	HBD c.49G→C		Surinam
11	Thalassemia	1.3	0.8	Not visible	Not visible	HBD c.82G→T		Surinam
12	Thalassemia	1.7	0.5	Not visible	Not visible	HBD c.82G→T	$-\alpha^{3.7}$	Iran
13	Thalassemia	1.8	0.5	Not visible	Not visible	HBD c.82G→T		Turkey
14	Thalassemia	1.6	0.5	Not visible	Not visible	HBD c.82G→T	$--\alpha^{Med\ I}$	Turkey
15	Thalassemia	1.4	0.4	Not visible	Not visible	HBD c.257T→C		Turkey
16	Thalassemia	1.5	2.3	Not visible	Not visible	HBD c.275dupT	HBG2 c.-90A→T	The Netherlands
17	Hb variant	2.1	0.4	Not visible	Slow HbX	HBD c.431A→G	HBB c.20A→T	Turkey
18	Hb variant	2.0	0.7	Not visible	Slow HbX	HBD c.431A→G	HBB c.20A→T	Turkey
19	Hb variant	2.1	0.4	Not visible	Slow HbX	HBD c.431A→G	HBB c.20A→T	Turkey
20	Thalassemia	2.0	1.3	Not visible	Not visible	HBD c.-118C→T		Surinam

(:HbVar nomenclature is reported in the text).*

β-thalassemia heterozygosity when this is based on the HbA_2 level only.

HbA_2-Etolia. The δ-thalassemic phenotype observed in one patient was caused by HbA_2-Etolia heterozygosity [*HBD* c.257T→C] (δcd85 TTT→TCT) (Figure 2C).[6] Because of the instability of HbA_2-Etolia, HbA_2 expression is decreased in the heterozygous state (1.4%) and no HbA_2 variant is visible on HPLC (Figure 1B). This may compromise the diagnosis of a β-thalassemia carrier.

HBD c.275dupT. One of the samples showed the heterozygous presence of a frame shift. We found the insertion of thymidine at c.275 (δcd 91) in the second exon of the δ-globin gene (Figure 2D). This mutation was reported once before in a Belgian family with δ^0-thalassemia in 1989.[7] The frame shift results in a premature stop of δ-globin synthesis at c.278 (δcd 94). In the present case the frame shift was found in a Dutch patient and resulted in a lowered HbA_2 level (1.5%) and an elevated level of HbF (2.3%). This patient was also found to be carrier of a new polymorphism on the ^{G}-γ globin gene [*HBG2* c.-90A→T](γ -37 A→T) apparently associated with elevated HbF expression.[8]

The presence of this δ-globin gene mutation, resulting in decreased HbA_2 expression, could compromise the diagnosis of β-thalassemia carriership.

HbA_2-Abruzzo. In one family HbS occurred with slightly decreased levels of HbA_2 (2.0%) and an additional slow moving HbA_2 fraction on electrophoresis. After sequencing we found a heterozygous mutation at c.431 (δcd 143). Histidine replaced arginine due to a CAC→CGC transition (Figure 2E). Further family research revealed that the δ-variant was not localized on the same chromosome as the HbS mutation. The mutation has an equivalent on the β-globin gene, called Hb-Abruzzo, which has been found in a few Italian and Italian-American families.[9] This δ-variant also causes a risk of overlooking β-thalassemia carrier ship when a diagnosis is based on the HbA_2 level.

HBD c.-118C→T. In one of the samples we found a new mutation at position c.-118 (δ -68) (Figure 2F). The mutation C→T is located in an AACCAAC sequence (c.-120 to -114)(δ -70 to -64) in which another δ-thalassemia mutation has been reported (*HBD* c.-115A→G)(δ -65A→G).[10] The sequence is considered to be a regulatory element, like the AAC-

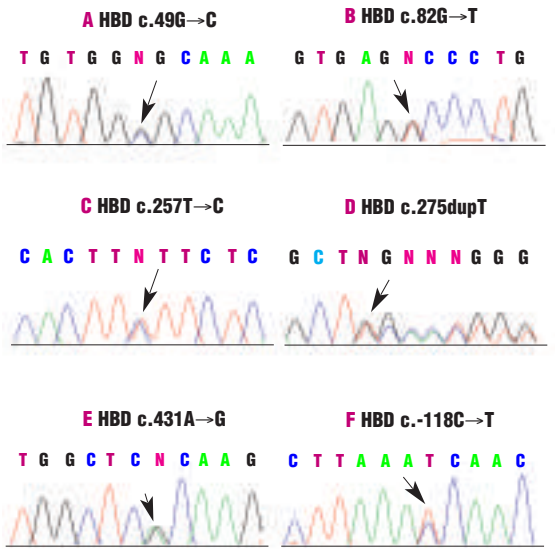

Figure 2. Sequence results of the δ-globin gene mutations found.

CAAT sequence, which normally exists in the β-globin gene. CCAAT sequences are critical in all globin genes, and many tissue-specific genes, for the correct initiation point and a high level of transcription. The modification of CCAAT to CCAAC is considered to be responsible for the lower transcription level of the δ-globin gene in respect to the β gene, which emphasizes the remarkable effect of a single nucleotide substitution in these conserved sequences.[11] Due to the A→G substitution at position -115 (δ -65)(resulting in AACCAGC) the transcription factor GATA-1 has a decreased binding ability. Accordingly, our patient had a slightly decreased HbA_2 level (2.0%). The presence of the *HBD* c.-118C→T mutation could compromise the diagnosis of β-thalassemia carriership, because of the lower HbA_2 expression.

In conclusion, over 600 β- and only about 60 δ-globin gene mutations have been reported so far [*http://globin.cse.psu.edu/hbvar/menu.html*]. It is quite likely that the number of mutations on the δ-gene is comparable to the number of mutations on the β-gene. Therefore, more attention should be paid during diagnostics to a possible (co-) inheritance of a δ mutation, because, though not pathologically significant, the knowledge of these mutations could avoid a misdiagnosis of β-thalassemia minor based on HbA_2 levels.

MJB, a graduate in Biomedical Science, is participating in several ongoing projects at the Hemoglobinopathies Laboratory and was in charge of this study; CLH, Staff Member and Molecular Geneticist was consultant in this. PVD, Senior Technician provided practical assistance. PCG, Head of the Laboratory, coordinated the study and supervised the writing.

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