

## **Comparison of two known chromosomal rearrangements in the $\delta\beta$ globin complex with identical DNA breakpoints but causing different Hb A<sub>2</sub> levels**

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## *Abstract*

We report three cases with very heterogeneous HbA<sub>2</sub> levels caused by known chromosomal rearrangements in the  $\beta$ -globin locus. These rearrangements had their breakpoints at the same region in the  $\delta$ -gene, leading either to the  $\delta^0\beta^+$ -Senegalese deletion or to an insertion of a  $\delta$ -gene, known as Anti-Lepore. One patient showed, apart from drastically increased HbA<sub>2</sub> values of 17%, inconspicuous hematological values. He had an Anti-Lepore mutation with three copies of the  $\delta$ -gene explaining the high HbA<sub>2</sub> level. Two other patients had HbA<sub>2</sub> levels in the lower borderline range and increased HbF levels. Molecular analysis showed the  $\delta^0\beta^+$ -Senegalese deletion. One of them presented with an additional mild  $\beta$ -thalassemia mutation leading to thalassemia intermedia. These cases illustrate that different gene rearrangements with the same breakpoints in the delta gene can lead to different levels of HbA<sub>2</sub> depending on the number of  $\delta$ -genes remaining.

## *Key words*

hemoglobin, thalassemia, gene rearrangement, HbA<sub>2</sub> levels,  $\beta$ -globin gene cluster

## *Running head*

Gene rearrangement in the  $\beta$ -globin complex

Normal Hemoglobin A<sub>2</sub> levels range between 2.1-3.2% of total hemoglobin (Hb) (1). Low HbA<sub>2</sub> values reflect different abnormalities: most frequently iron deficiencies,  $\alpha$ -thalassemia ( $\alpha$ -thal) or  $\delta$ -globin abnormalities, which can either be structural  $\delta$ -globin variants or  $\delta$ -thal. Slightly increased HbA<sub>2</sub> can be caused by vitamin B12 & folate acid deficiencies, hyperthyroidism or antiviral treatment (2). An increased HbA<sub>2</sub> value between 3.5% and 6% is seen in most  $\beta$ -thal carriers and thus is the most important diagnostic marker of  $\beta$ -thal minor. If the  $\beta$ -thal is caused by some deletions comprising the 5' promotor, the increase in HbA<sub>2</sub> can be as high as 8% to 12% (3).

Here, we compare three cases with known chromosomal rearrangements that all have identical breakpoints in the  $\delta$ -gene. They illustrate that the HbA<sub>2</sub> level can vary dramatically depending on the number of functional  $\delta$ -genes being present.

#### *Increased HbA<sub>2</sub> levels due to three copies of the $\delta$ gene*

*Case 1:* A 50-year old man from South East Asia was referred for HbA<sub>1C</sub> measurement as diabetes mellitus control. Hb analysis was performed using cation exchange high performance liquid chromatography (HPLC, VARIANT II™; Bio-Rad Laboratories, Hercules, CA, USA) and showed 17% HbA<sub>2</sub>. The identity of HbA<sub>2</sub> was further confirmed by capillary electrophoresis (Sebia) and isoelectric focusing (4).

The hematological values of this patient were normal (Table 1). In order to investigate whether this drastic increase in HbA<sub>2</sub> could result from gene rearrangement in the  $\beta$ -globin region a polymerase chain reaction (PCR) with the primers A1 and A2 described by So *et al.* (5) was performed. The sequence of the amplified fragment was homologous to the  $\beta$  promoter at the 5'-end and to the  $\delta$  open reading frame (ORF) at the 3'-end. The breakpoint was in a 54 bp window between the cap site and codon 8, corresponding to the  $\beta\delta$  fusion gene anti-Lepore Hong Kong (NG\_000007.3:g.(70570\_70625)insNG\_000007.3:g.(63154\_63209)\_(70570\_70625)) as described (5). The reported HbA<sub>2</sub> levels of around 16-18% in the heterozygous state correspond well to the values found in our patient.

#### *Decreased HbA<sub>2</sub> levels due to one copy of the $\delta$ gene*

*Case 2:* A 43 year old Egypt woman living in Switzerland had no clinical history and gave birth to three healthy children. A few weeks after insertion of a copper contraceptive coil, she suffered from hypermenorrhea. Upon Venofer® infusion the ferritin level temporarily raised

to 104 mg/l but dropped again below 40 mg/l. The hematological indices are comparable to heterozygous  $\beta$ -thal (Table 1). HPLC (Poly CAT A, Poly LC Inc., Columbia, MD, USA) revealed a HbA<sub>2</sub> level in the lower normal range and an increased HbF of 14%. Multiplex ligation probe amplification (MLPA kit P102-B1 HBB) showed a deletion of the  $\delta$ -globin gene coding sequence, the  $\delta\beta$ -intergenic region and also the  $\beta$ -globin gene promoter. To determine the deletion breakpoint, a GAP-PCR reaction was performed with primers E1/E3 described by Thein *et al.* (6). Sequence analysis revealed that the deletion had occurred exactly in the same window as observed for case 1. However, whereas in case 1 the chromosome rearrangement resulted in an additional  $\beta\delta$ -fusion gene, here the rearrangement caused a deletion of the  $\delta$ -ORF, joining the  $\delta$ -promoter directly to the  $\beta$ -ORF. This deletion has already been described by Zertal-Zidani *et al.* (7), is 7.4kp long and is named Senegalese  $\delta^0\beta^+$ -thalassemia (NG\_000007.3:g.(63154\_63209)\_(70570\_70625)del7417).

*Case 3:* A 34-year old Algerian male living in Switzerland suffered from thal-intermedia with massive splenomegaly but moderate anemia (Table 1). He was not transfused so far. HPLC revealed a slightly reduced HbA<sub>2</sub> level of 1.9% and significantly increased HbF (74%). Sequencing showed a homozygous point mutation in the promotor region of the  $\beta$ -gene, HBB:c.-29A>G. This mutation causes increased HbA<sub>2</sub> levels in both the heterozygous or homozygous state (8). However, here we observed a reduced HbA<sub>2</sub> of only 1.9%. We therefore analysed the  $\beta$ -globin complex by MPLA. Interestingly, we found the  $\delta^0\beta^+$ -Senegalese deletion as in case 2. Thus, in both patients carrying this deletion, only one functional  $\delta$  gene is present, which explains the reduced HbA<sub>2</sub> levels.

In hemoglobin analysis the level of HbA<sub>2</sub> plays a critical role in identifying the type of hemoglobin anomaly. Here, we compare three cases with either increased or decreased HbA<sub>2</sub> levels due to chromosomal rearrangement. In the first case we identified a heterozygous anti-Lepore Hong Kong with HbA<sub>2</sub> of 17%. Molecular analysis revealed that in this proband three intact copies of the  $\delta$ -gene are present, two being driven by the  $\delta$ -promoter and one by the  $\beta$ -promoter. Considering that the  $\beta$ -gene is expressed at much higher levels than the  $\delta$ -gene in a wild type situation, it is surprising that we only observe 17% of HbA<sub>2</sub>. It has been shown that the  $\beta$ -promoter is stronger than the  $\delta$ -promoter due to the CACCC and CAATT conserved regions (9, 10). Also, genes that are more proximal to the 5' LCR (locus control regions) seem to have a transcriptional advantage over more distant genes (11). This supports the observation that the beta-delta fusion gene is expressed at higher levels resulting in an

increase of HbA<sub>2</sub>. On the other hand, it has been debated that the  $\beta$ -IVS 2 region is critical for the high expression level of the  $\beta$ -gene and that the  $\beta\delta$ -fusion mRNA is less stable than the  $\beta$ -mRNA, which would explain the moderate increase in HbA<sub>2</sub> (12).

For case 2 and 3 gene rearrangement results in loss of one  $\delta$  gene. This explains the unexpectedly low level of HbA<sub>2</sub> observed in both cases. The high HbF level in case 3 on the other hand can be explained by the additional thalassemic -29A>G mutation, leading to thal-intermedia.

The chromosomal rearrangements described here are very likely the result of unequal cross-over between misaligned copies of chromosome 11 during meiosis. It can either result in addition of a  $\delta\beta$ -fusion gene as observed for case 1 or in the removal of one copy of the  $\delta$ -gene as for case 2 and 3. Another possible mechanism of the present rearrangements is replication slippage (13). Apparently, the direct repeats where the breakage occurs need to be of a certain length to mediate unequal crossing over. For shorter direct repeats - as described here - replication slippage could take place. The key feature of this model is that during replication, the primer and template strands can transiently dissociate and then re-associate in a misaligned configuration. If two direct repeats are located on the same chromosome one can imagine the following scenario: If the newly synthesized second repeat slips backwards to mispair at the first repeat, continued DNA synthesis results in a duplicational insertion as seen for anti-Lepore Hong Kong. If the newly synthesized first strand slips forward to mispair at the second repeat, continued DNA synthesis results in a deletion as seen for the  $\delta^0\beta^+$ -Senegalese deletion (13).

Noteworthy, chromosomal rearrangements in the  $\delta\beta$ -globin region not only occurred in human beings but also in other mammals. In the African elephant *Loxodonta Africana* for instance the presence of a chimeric  $\delta\beta$ -fusion gene has been reported (14). The situation in the African elephant is unique in that the chimeric  $\delta\beta$ -fusion gene replaces the parental HBB gene and is therefore solely responsible for synthesizing the  $\beta$ -chain subunit of adult hemoglobin. In muroid rodents, a chimeric  $\gamma\epsilon$ -fusion gene was created by unequal crossing over between the embryonic  $\epsilon$ - and  $\gamma$ -genes. Interestingly, this  $\gamma\epsilon$ -fusion gene was generated in the same fashion as the anti-Lepore Hong Kong in humans (15).

The rearrangements discussed above have been described before as anti-Lepore Hong Kong and the  $\delta^0\beta^+$ -Senegalese deletion, respectively (5, 7). However, this is the first report comparing these two possible gene rearrangements on a molecular and hematological level. Additionally, case 3 demonstrates the clinical impact of a combination with the -29A>G  $\beta$ -

thal mutation. This patient presents with  $\beta$ -thal intermedia with massive splenomegaly. The proband has never been transfused to date. A similar case with a compound heterozygosity for the -30T>A  $\beta$  thalassemia mutation and the  $\delta^0\beta^+$  Senegalese deletion however, was shown to present with a transfusion-dependent form of  $\beta$ -thal intermedia (16).

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*Declaration of interest*

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.



*Table legend:*

Table 1: Hematological, biochemical and molecular data of cases 1 to 3.