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Title: Toward prevention of Hemoglobinopathies in Oman

Issue Date: 2015-09-22

CHAPTER

HB LANSING AND A NEW β PROMOTER
TRANSVERSION (- 52 G>T): AN ATTEMPT
TO DEFINE THE PHENOTYPE
OF TWO MUTATIONS FOUND
IN THE OMANI POPULATION

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Hemoglobin. 2015;39(2):111- 4



ABSTRACT

We report two examples showing how problematic it can be to define the phenotype of new or rare globin genes mutations. We describe two mutations observed for the first time in Omani: The first has been found in the consanguineous parents of a deceased newborn with hepatomegaly, cardiomegaly and severe hemolytic anemia, putative homozygous for the rare Hb-Lansing ($\alpha 2$ cd87 CAC>CAG). The second is a novel β -globin gene promoter mutation (-52 G>T) observed in four independent patients. Two with borderline/elevated HbA₂, α -thalassemia and hypochromic red cell indices and two with HbS heterozygosis, alpha thalassemia and with HbA / HbS ratios possibly indicating a very mild β^+ thalassemia mutation.

INTRODUCTION

Stable and well expressed α globin chain variants have usually no clinical consequences. Those unstable or thalassemic, although nearly asymptomatic in the carriers, may present with a more severe phenotype in the homozygous state (1) or in association with α -thalassemia mutations, while those with abnormal oxygen affinity may lead to polycythemia, cyanosis, tissue hypoxia or respiratory distress (2).

Homozygosis for rare hemoglobin (Hb) variants is rarely observed and generally in cases of consanguinity while combinations with thalassemia or with common variants can be found more frequently in endemic areas. We present the case of a newborn with hepatomegaly, cardiomegaly and severe hemolytic anemia who died shortly after birth. Although examination of the propositus at the molecular level was not possible, due to the presence of a rare Hb variant in both consanguineous parents, we presume that this could be the first described case of homozygosis for Hb Lansing.

Screening couples for premarital prevention of Hemoglobinopathies is done in Oman by routine hematology and high-performance liquid chromatography (HPLC). The common Hb variants will then be putatively recognized while an elevated HbA₂ will diagnose the β -thalassemia carriers. Borderline HbA₂ measurements can, if disregarded, lead to the misdiagnosis of normal HbA₂ β -thalassemia traits and further investigation at the molecular level might be necessary to avoid mistakes and to check for unexpected mutations. In this report, we describe the characterization of a novel β^+ promoter determinant associated with borderline HbA₂ found in four independent cases and we report how the expression of the mutated allele has been assumed.

MATERIALS AND METHODS

The putative Hb Lansing homozygosity

[$\alpha 87(\text{F8})\text{His} \rightarrow \text{Gln}$; $\text{CAC} > \text{CAG}$ (HBA2 : c.264C4G)]

After the death of a newborn, blood was collected in EDTA from the consanguineous parents. A complete blood count (CBC) was performed and was analyzed by high performance liquid chromatography using the VARIANT II_ (Bio-Rad Laboratories, Hercules, CA, USA), as previously described (3). Genomic DNA was extracted from whole blood, using the Qiagen kit as per the manufacturer instructions. Alpha-globin genotype was established by GAP-PCR for the seven most common alpha thalassemia deletions (4). The α_2 - and α_1 -globin genes were sequenced using an ABI Prism 3730 DNA sequencer (Applied Biosystems, Perkin Elmer Corporation, Foster City, CA, USA) as previously described (5). Due to blood transfusion directly after birth, no HPLC was performed on the new born, neither was material made available for further DNA studies. This was the first child born to the consanguineous couple.

The putative β^+ -thalassemia mutation [-52 (G>T)]

Blood was collected in EDTA during premarital screening from four independent Omani individuals. Hematological data were obtained from a reference Hematology laboratory. Due to borderline HbA₂ values in two cases and the presence of HbS (HBB: c.20A>T) in the other two cases, molecular investigation was undertaken using the same methodology described above.

RESULTS

Case one

The infant was born after 37 weeks of gestation. The parents (first cousins) originated from southern Oman. Covered with a thick meconium, the newborn, presented in respiratory distress with low oxygen saturation ($\text{SpO}_2 < 50\%$). In spite of resuscitation attempts the newborn died shortly after birth. The clinical description was pulmonary hypertension, hypoxia, hepato- and cardiomegaly, multi-organ dysfunction and severe anemia. Due to urgent transfusion no blood was collected and no material was made available for further investigations.

Parent's analysis revealed normal hematology (Table 6.1) and unclear HPLC patterns with low HbA_2 levels (data not shown). DNA analysis revealed none of the common alpha thalassemia deletions but sequencing showed heterozygosity for Hb-Lansing [$\alpha 2$ (F8) His>Gln Cd87 CAC>CAG (HBA2:c.264C>G)] in both healthy parents.

Table 6.1. Hematological and molecular data of the parents heterozygous for Hb-Lansing (SpO_2 was not measured in the parents).

	Mother	Father
HbA%	84.9	84.3
HbA₂%	2.1	2.3
HbF%	0.4	0.6
α- genotype	$\alpha\alpha^T/\alpha\alpha$	$\alpha\alpha^T/\alpha\alpha$
Hb (g/dl)	14.3	15.9
RBC	4.9	5.4
MCV (fl)	87.1	87.5
MCH (pg)	29	29.3

Case two

All four cases, carriers or non-carriers of HbS, presented with low-normal Hb levels and microcytic hypochromic parameter which is not unusual in Oman due to the high frequency of α -thalassemia. The lowest Hb and MCV were measured in a male homozygous for the $-\alpha^{3.7}$ alpha thalassemia deletion with nevertheless a borderline HbA_2 of 3.6%. The second non-carrier of HbS was a female heterozygous $-\alpha^{3.7}$ with a border line MCV and a slightly elevated HbA_2 level of 3.9%.

The two HbS carriers, both females and carriers of the $-\alpha^{3.7}$ deletions, presented with an HbS expression higher than expected for their genotype combination. Their HbA_2 level was decisively elevated (4.1 and 5%) but unreliable because of the overlapping HbS1c.

DNA analysis of the *HBB* gene in the two independent non HbS carriers showed a normal sequence except for the heterozygous state for a G→T transversion at position -52 relative to the Cap site (Figure 6.1). In the other 2 subjects, carriers of HbS, DNA sequencing revealed the same -52 (G>T) transversion and confirmed the HbS mutations. Data are summarized in Table 6.2.

Table 6.2. The hematological and molecular profiles of 4 independent subjects all carriers of the -52 mutation.

β -genotype	age/ gender	HbA %	HbA ₂ %	HbS %	HbF %	Hb (gm/dl)	MCV (fl)	MCH (pg)	α -thal genotype
β^{-52}/β^A	4y/M	84.1	3.6	–	1.9	10.8	58.8	18.6	$-\alpha/-\alpha$
β^{-52}/β^A	27y/F	86.6	3.9	–	0.6	12.4	82.6	24.5	$-\alpha/\alpha\alpha$
β^{-52}/β^S	28y/F	45.5	4.1	44.1	2	ND	ND	ND	$-\alpha/\alpha\alpha$
β^{-52}/β^S	20y/F	55.7	5	38.6	0.7	12.6	69.5	22.2	$-\alpha/\alpha\alpha$

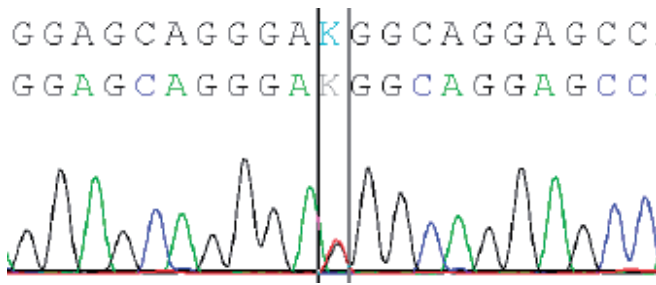


Figure 6.1. DNA sequence electropherogram of the β -globin promoter region showing heterozygosity for the -52 (G>T) nucleotide transversion.

DISCUSSION

Case one

This is the first report of a presumed Hb Lansing homozygosis. In spite of the lack of a molecular confirmation and of the fact that the condition of the newborn could be caused by other unknown congenital conditions (of which we have no knowledge or evidence), we presumed that the phenotype we have observed could be caused by homozygosis for Hb Lansing. If this is indeed the case and if the variant is relatively frequent in Oman, homozygosis could account for other cases of unexplained neonatal mortality in the country.

Hb Lansing, firstly reported by Sarikonda et al. as unstable but visible in carriers (6), was not measurable or visible in the mother and father of our propositus using routine HPLC (Figure 6.1).

In the original description Hb Lansing was observed in a 24-year-old asymptomatic Hispanic man (6) with SpO₂ of 88% and normal parameters (Hb =13.7 g/dl; HCT = 40.1%; reticulocyte count = 1.6%). These authors reported an abnormal fraction of 10-12% eluting “in front of HbA” on HPLC. Such an expression does not fit with our results and with the severe condition we have seen in our presumed homozygous and could be the honest report of a methemoglobin fraction which is regularly seen in front of HbA when running conserved samples on HPLC. More compatible with our observations seems to be the expression reported by Ishitsuka et al. in a 52-year-old Asian woman heterozygous for Hb-Lansing with a

low SpO₂ (83–86 %) with the following parameters: Hb = 13.3 g/dl; HCT = 40.2 %; MCV = 87.0 fl and reticulocyte count = 70,000/L (7). These authors show in their Fig. 1b the electrophoretic separation of Hb Lansing in which a band of approximately 1% is visible on position F in a further normal separation.

The proximal histidine α 87 residue (F8) anchoring the heme is critical for the molecule structure. Substitution of this residue is bound to cause instability and loss of function (8). Four other mutations have been reported at codon 87 resulting in other hemoglobin variants. The unstable Hemoglobin Iwata variant (His > Arg) was first identified in a healthy Japanese carrier with slight reticulocytosis (9). Hemoglobin M-Iwate (His > Tyr), a methemoglobin which decreases oxygen affinity and leads to cyanosis (10). Hemoglobin Auckland (His > Asp) which causes molecular instability and heme loss leading to mild hemolytic anemia (8). Finally, Hemoglobin Grifton (His > Pro) leading to microcytosis without clinical phenotype in the carrier (6).

In our case, Hb Lansing was not disturbing the hematological parameters of the consanguineous parents. However, since no other explanations have been found for the clinical symptoms of the newborn we could assume that the infant could have inherited 2 copies of the Hb Lansing mutation and that this genotype could be accounted for the severe intrauterine condition. If this is indeed the case, diagnose before conception and intrauterine transfusion could have kept the fetus in reasonably good conditions until birth. The question is how severe would have been the postnatal condition and our conjecture would be a severe transfusion dependent HbH disease like phenotype, similar to homozygosis for Hb Constant Spring (11). Mutations affecting the α_2 gene are generally more severe as they normally account for approximately 2/3 of α -globin chain synthesis (12). Low SpO₂ in healthy patients could be due to hemoglobin variants and care should be taken when low SpO₂ levels are detected (13).

Case two

Routine diagnosis of beta-thalassemia trait is based on microcytic parameters and elevated levels of HbA₂ measured using dedicated high performance liquid chromatography (HPLC) or capillary electrophoresis (CE) devices. Although very precise, these machines are subject to some inevitable variability and cutoff values should never be taken for granted (14). While values above 4% are in general fairly diagnostic, “grey values” between 3.5 and 4% are not and need to be investigated further. Mutations in the promoter region of the beta globin, the evolutionary conserved sequences responsible for transcription regulation, have been reported to be associated with relatively mild forms of β -thalassemia (15, 16). These motifs includes the CACCC boxes at nt -105 to -101 and -90 to -86 from the cap site (17), the CCAAT box at -76 to -72, and the TATA box at -30 to -26.

A common mutation on the promoter region with HbA₂ values around 3.5% is the C>T transition at -101 (18). Another silent promoter mutation (-71 C>T) was recently reported in Omani (19) while another promoter region known as direct repeat element (DRE) has been suggested to play a role in β -globin transcriptional regulation and found to be an important regulatory element required for maximum transcription levels from the β -globin promoter in erythroid cells in mouse (20). The novel -52 G>T trasversion described in this paper lies within the conserved DRE and other mutations in *HBB* DRE surrounding associated with β -thalassemic

have been described by Li DZ et al (-50 G→A) (21) and Irengue et al. (-42 C→G) (22). To the best of our knowledge the present case is the third report of a mutation in the DRE region. The question rises is this mutation a silent polymorphism or a mild β^+ thalassemia determinant?

Due to the presence of alpha thalassemia very common in Oman (23), this question cannot be easily answered looking at the hematological phenotype of the plain carriers with borderline HbA_2 . Conversely, looking at the two cases compound heterozygous -52 and HbS we can observe the specific expression of the two alleles. Then, in the presence of $-\alpha^{3.7}$ heterozygosis the HbS expression should be around 30-35% while it is measured between 38 and 44% in our cases. The last measurement in particular could indicate that the -52 mutation is not on the HbS allele and that at least a 10% reduction in the expression of the -52 would probably cause a potential >50% HbS expression in absence of α -thalassemia. Based only on these calculations, the clinical impact of the -52 mutation seems to be very mild. On the other hand one needs to see the results of a genotype combination with a β^0 thalassemia mutation to exclude for 100% the risk of a (mild) thalassemia intermedia. Moreover, it is important to mention that Hb S-Oman (*HBB*: c.[20A>T;364G>A]) is a more severe sickling double mutation on the same β chain, which clinically manifests as a moderate hemolytic anemia in carriers with only 20.0% Hb S, and that the impact of the -52 mutation in combination with Hb S-Oman could be more significant than a plain -52/Hb S compound heterozygosity (24).

Take home message

Although rarely observed in homozygous state, rare variants may come together in the progeny of consanguineous partners and one can expect to see rare variants in homozygous form or in combination with common variants or β and α thalassemia more often in Oman than elsewhere. If Hb Lansing, would appear to be relatively frequent in Oman, and if indeed, the presumed severe phenotype associated with homozygosis is correct, the condition could account for other cases of unexplained neonatal mortality in the country.

Knowledge on the variant's clinical implications and predicting the underlying pathology is important for genetic counselling but not always uncomplicated and molecular analysis for couples presumed at risk becomes essential if unstable variants cannot be detected at the hematological level. As carriers of Hb Lansing are asymptomatic and practically undetectable on HPLC, one should consider adding SpO₂ to the premarital screening protocol in isolated communities with consanguineous traditions where Hb Lansing is known to occur.

Likewise, one can expect mild β^+ thalassemia alleles with normal or slightly elevated HbA_2 levels such as -52 G>T to combine with severe β^0 -thalassemia mutations possibly causing intermediate conditions.

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