

Hemoglobin Interlaken in combination with beta thalassemia trait

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Abstract

We report a rare $\alpha 1$ globin gene variant (Hb Interlaken) found in a 63-year-old woman of Italian ancestry living in Buenos Aires Province, Argentina. The variant, a missense mutation at cd15 (GGT \rightarrow GAT) causing a Gly \rightarrow Asp amino acid substitution and also known as Hb J Oxford, was found in combination with the common thalassemia trait cd 39 (C \rightarrow T). The clinical picture of the patient was that of a β -thalassemia trait.

Introduction

In 1964 Liddell first described hemoglobin Interlaken in an English family.¹ Only isolated reports exist in which the same mutation was named Hb J-Oxford or Hb N-Cosenza.² The variant has thus far been described in combination with β -thalassemia homozygosity and HbS but not in combination with β -thalassemia trait and never before in Argentina, a multi ethnic country with many ethnic components.

Hb Interlaken is a stable $\alpha 1$ chain variant caused by a GAC \rightarrow CAC transversion at codon 15 of the $\alpha 1$ gene causing a Gly \rightarrow Asp amino acid substitution (HGVS nomenclature HBA1:c.47G>A).³

Case Report

We report the identification of Hb Interlaken in a 63-year-old Argentinian woman of Italian ancestry, referred to our laboratory because of a microcytic hypochromic anemia. Hb Interlaken was found in this patient in combination with the common Mediterranean β^0 tha-

lassemia trait cd 39 (C \rightarrow T) (HGVS nomenclature HBB:c.118C>T), the most frequent β -thalassemia mutation in Argentina.⁴

Complete blood count (CBC) was obtained with a Coulter Counter model ACT10 (Beckman Coulter Inc, Brea, CA, USA): Hb (g/L) 11.6, RBC ($10^{12}/L$) 5.88, MCV (fL) 62.4, HCM (pg) 19.7, reticulocytes (%) 1.0.

The separation of the Hb fractions was done on alkaline cellulose acetate electrophoresis (Figure 1A). The presence of a J like minor fraction was suggestive for an α chain variant. Hb A2 measured by elution from electrophoresis followed by spectrophotometric measurement of the absorbance at 415 nm was estimated at 3% while Hb X was 28%. In spite of the normal HbA₂ level, the CBC, the slightly elevated HbF (1.3%) estimated according to Betke *et al.*^{5,6} indicated a possible β -thalassemia trait while the very low mean corpuscular hemoglobin could be an indication for a β - α thalassemia combination. Iron parameters were measured as previously described:⁷ serum iron 56 μ g/dL, total iron-binding capacity 328 μ g/dL, transferrin saturation 17% and serum ferritin 4 ng/mL.⁸ Isopropanol,⁹ heat stability and sickle tests were performed and were all negative.

DNA was extracted from peripheral blood sample.¹⁰ Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR)¹¹ was used to confirm the presence of the $\beta^0 39$ mutation (*data not shown*).

Alpha thalassemia deletion analysis was done using Gap-PCR¹² taking into account the ethnic origin and hematological data of the patient while for point mutation analysis amplification of the $\alpha 2$ - and $\alpha 1$ -globin genes was performed as previously described by using oligonucleotide primers (CyberSyn, Lenni, PA, USA); the common forward primers Faa2: 5'-CGCGCTCGCGCCCGGCAC-3', and reverse specific primers for the $\alpha 2$ gene: 5'-GGGAGGCCATCGGGCAGGAGGAAC-3' and $\alpha 1$ gene: 5'-GGGGGGAGGCCAAGGGGCAAGAA-3'. Sequencing was done using a Big Dye Terminators Ready Reaction Kit (Perkin-Elmer Cetus, Norwalk, CT, USA) in an ABI PRISM 310 sequencer (Perkin-Elmer Cetus). Primers used for sequencing the two genes were the following: exon 1, common forward primers Faa2; exon 2, primer S2 (5'-CCC GCCGGACCACA-3'); exon 3, primer S3 (5'-GCCGGTTGCGGGAGGT-3'). The reverse specific primers for the $\alpha 1$ gene were used to confirm the mutation.

GAP-PCR were used to detect α^0 deletions (α)20.5, —MED and α^+ deletions $-\alpha^{3,7}$ and $-\alpha^{4,2}$ and were all negative. Sequencing of $\alpha 1$ gene revealed a GAC \rightarrow CAC (Asp \rightarrow His) substitution at codon 15, corresponding to Hb Interlaken (Figure 1B).

Even though Hb Interlaken is detectable by electrophoresis a correct characterization of

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Key words: thalassemia, hemoglobinopathies, microcytic hypochromic anemia, Hb Interlaken.

Acknowledgments: we are very grateful to Dr M.C. Rosatelli from the Istituto di Clinica e Biologia dell'Età Evolutiva, Università degli Studi Cagliari, Italy who provided DNA samples with known mutations.

Contributions: IMB, MJO, conception and design; IMB, SMP, MJO, BE, GC, AFP, MEV, development of methodology; IMB, GC, BE, acquisition of data; IMB, SMP, MJO, KLC, GC, BE, AFPratti, MEV, analysis and interpretation of data; IMB, SMP, MJO, KLC, ACM, writing, review and/or revision of the manuscript; IMB, KLC, AFP, MEV, ACM, administrative, technical, or material support; IMB, SMP, MJO, ACM, study supervision.

Conflict of interests: the authors report no conflict of interests.

Received for publication: 10 October 2012.

Revision received: 14 December 2012.

Accepted for publication: 7 January 2012.

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Thalassemia Reports 2013; 3:e3
[doi:10.4081/thal.2013.e3](https://doi.org/10.4081/thal.2013.e3)

the genotype requires better methods, especially in combination with α thalassemia. Then more sophisticated systems able to measure Hb fractions more precisely like high-performance liquid chromatography or capillary electrophoresis¹³ are needed before molecular analysis.

In our case the estimation of the HbA₂ that should have been elevated was typically normal risking misdiagnosis. The underestimation was due to the fact that part of the delta chain is bound by the mutated α chain and this abnormal HbA₂ fraction migrates on a different spot and is lost for measurement. Therefore the measured HbA₂ value (3%) should be augmented by 28% resulting into a 3.84% a still ambiguous but elevated HbA₂.⁶ Even though our value (28%) for hemoglobin variant J-Oxford is high, the consulted references showed values between 21 and 25%.¹⁴⁻¹⁶

It is the first time that this abnormal hemoglobin is described in our country. The low red blood cell indexes observed in this case are due to co-inheritance of β^0 thalassemia and the underestimation of HbA₂ could be caused

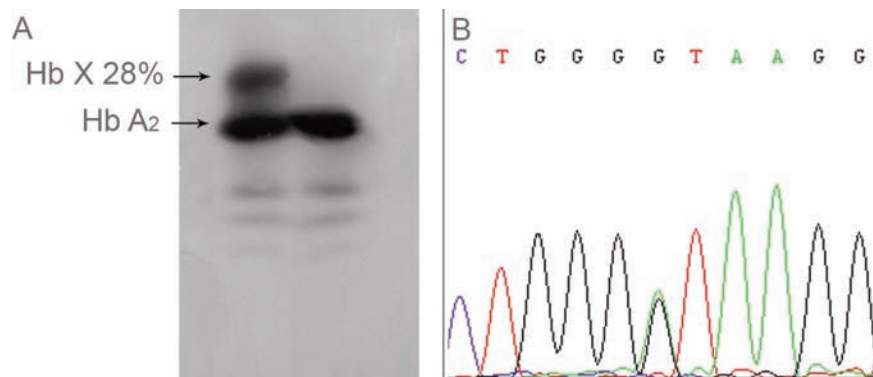


Figure 1. A) Hemoglobin electrophoresis at alkaline pH. B) Sequencing of the $\alpha 1$ -globin gene showed one mutation corresponding to Hb Interlaken (Hb J-Oxford, Hb N-Cosenza), [$\alpha 15\text{Gly} \rightarrow \text{Asp}$, GGT>GAT].

by iron deficiency in the patient at the time of diagnosis and lost of the abnormal HbA₂ fraction.

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