

Hb Southampton [B106(G8)Leu→PRO, CTG→CCG] in a Uruguayan woman

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Hemoglobin Southampton (also known as hemoglobin Casper) is a rare hemoglobin structural variant resulting from a substitution of a leucine residue for proline at codon beta106 [beta106(G8)Leu→Pro, CTG→CCG]. It is very unstable and associated with severe hemolytic anemia. We detected this mutation in a 37-year-old Uruguayan woman with a history of severe chronic hemolytic anemia since her childhood. According to our knowledge this is the first time that this variant has been found in the Uruguayan population.

Keywords: Hemoglobins; Hemoglobinopathies; Anemia, hemolytic, congenital; Humans; Female; Adult; Case reports

Introduction

Unstable hemoglobins (Hbs) are structural variants mainly characterized by single amino acid substitutions in the globin chain, but occasionally may be due to amino acid insertions or deletions and truncated or extended globin chains. These hemoglobins are commonly associated with autosomal-dominant hereditary hemolytic anemia produced by the formation of the inclusion of precipitated denatured hemoglobin called Heinz bodies in the red cell. The most common causes of instability are disruptions of the alpha helix of globin chains, alterations in the steric configuration of heme pocket and alterations in the tertiary structure of globin chains.

Here, we report a case of Hb Southampton (also known as Hb Casper) (b106 Leu→Pro, CTG→CCG) in a woman who was diagnosed for the first time at age 37 and referred to our laboratory with a history of severe chronic hemolytic anemia. This amino acid substitution (b106 Leu→Pro) alters the tertiary structure of the beta-chain in a region where there is direct contact with the heme group⁽¹⁾. This hemoglobin was first reported by Hyde et al.⁽²⁾, and then was reported in seven patients including two patients in the Argentines⁽³⁻⁸⁾, all due to *de novo* mutations.

Case report

A 37 year-old-woman from the city of Montevideo, Uruguay, was referred to our laboratory with a history of undiagnosed chronic hemolytic anemia since her childhood. The patient was born at term after a normal pregnancy and delivery. At four months of age she was prescribed iron due to anemia. At eleven months of age, she had a respiratory tract infection with anemia and jaundice. She was given her first transfusion at twenty-one months of age, when she had a new episode of respiratory tract infection with anemia and fever. Splenomegaly was detected at the time and she was misdiagnosed as beta thalassemia major based on her clinical data. The patient was continuously receiving blood transfusions until she was 16 years old (in 1990), when splenectomy was performed.

Physical examination revealed prominent skull, inferior maxilla and malar eminences as well as palpebral fissures sloping upwards and backwards, with jaundice. This phenotype was previously described in an Argentinian boy with Hb Southampton⁽⁷⁾.

Red blood cell indices were electronically determined with an automated cell counter (Cell Dyn 3700). The screening for the most common structural hemoglobinopathies (Hb S, Hb C) and the determination of the Hb A2 and Hb F levels were carried out in a Variant Hbs high-performance liquid chromatography (HPLC) system (BioRad).

DNA was extracted from a peripheral blood sample by standard methods. The seven most common deletional α -thalassemias (- α 3.7, - α 4.2, -SEA, -FIL, -MED, -(α)20.5, and -THAI) were checked by multiplex polymerase chain reaction (PCR)⁽⁹⁾. The non-deletional mutations (α Hpha, α NcoI and α aNcoI) were investigated by PCR and digestion with the restriction enzymes *HphI* and *NcoI*, respectively⁽¹⁰⁾. The beta-globin gene was amplified by PCR in two segments and sequenced in a ABI 3130 genetic analyzer (Applied Biosystems) according to conditions already described⁽¹¹⁾. The hematological data of the patient are shown in Table 1. The original microcytosis changed to macrocytosis possibly due to the deficit of folic acid and vitamin B12 caused by the continuous regenerative activity of the bone marrow tissue; the levels of folic acid and vitamin B12 were undetectable.

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Table 1 - Hematological data of the Hb Southampton carrier

	1980	1990	2002	2007
Hb (g/dL)	7.5	7.4	9.2	8.5
PCV (%)	20	20	29.3	28.9
RBC (x 10 ¹² /L)	2.9	2.16	2.77	2.44
MCV (fL)	68	93	105.7	118.3
MCH (pg)	25	29.6	33.1	34.8
RDW (%)			23.4	22.8
Hb A2 (%)		3.5		4
Hb F (%)		1.5		

Hb: hemoglobin; PCV: packed cell volume; RBC: red blood cell count; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; RDW: red cell distribution width

No abnormal Hbs were detected by the Variant Hbs HPLC system and no alpha-thalassemia mutations were found. However, sequencing of the beta-gene showed a base substitution (T→C) in the second position of the 106th codon leading to a Leu→Pro substitution in the beta-globin chain corresponding to Hb Southampton.

This study was conducted in accordance with the Helsinki declaration as revised in 2008. The patient gave written informed consent on participating in this study.

Discussion

Hb Southampton (b106 Leu→Pro, CTG→CCG) is the result of the substitution of leucine (located at position G8) by proline in the beta-globin. This substitution disrupts the alpha helix of the beta-chain and alters the tertiary structure of the hemoglobin molecule resulting in the loss of a heme group. This produces denaturation and precipitation of the hemoglobin molecule generating Heinz bodies which are associated with the red cell membrane and lead to premature cell destruction and as a consequence of chronic hemolytic anemia⁽¹²⁾.

It is unclear if the microcytosis originally observed in this patient is a characteristic of Hb Southampton. The patient reported by Eandi et al.⁽⁷⁾ presented microcytosis and hypochromia but not the patients reported by Avalos et al.⁽⁸⁾, the two patients reported by Koler et al.⁽³⁾ and the patient reported by Heintz et al.⁽⁶⁾. Therefore, the microcytosis originally observed may have been due to other conditions such as iron deficiency.

Although we do not have any samples from her parents to analyze, we can infer that this is a *de novo* mutation as previously reported⁽²⁻⁸⁾, because her parents did not present the clinical characteristics compatible with the presence of Hb Southampton. This variant can be detected by isoelectric focusing (IEF) but it is electrophoretically silent in conventional Hb electrophoresis, the method most commonly used in Uruguay. Also, the presence

of unstable hemoglobins, including Hb Southampton, can be suspected when screening tests such as isopropanol and the heat tests are positive as well as the presence of inclusion bodies by the brilliant cresyl blue technique⁽¹³⁾. These are inexpensive tests and easy to implement in any hematological laboratory.

Conclusion

Despite the widespread knowledge about the incidence and molecular basis of hemoglobinopathies, these diseases have not been considered a health problem until recently in Uruguay.

This report shows the necessity to update the methods used to detect hemoglobinopathies in Uruguay as well as to start considering these diseases as a health problem in our country in order to avoid unnecessary medical interventions.

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