Hb Nunobiki [α2 141(HC3) Arg→Cys; HBA2:c.424C>T] in Spain: Mutation de novo or Acquired?

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Abstract

The most common hemoglobin arose as an adaptation to the environment and its expansion was carried out by mechanisms of gene flow and population growth related to the social changes of habits of mankind. Other hemoglobinopathies (Hbs) to have low prevalence and appear in population studies of Hb or glycosylated hemoglobin (Hb A1c). Most of these Hbs have been identified in isolation and individually, others in a few families, considering these cases de novo mutations or that the mutated gene has been transported inadvertently. An example is Hb Nunobiki, discovered in Japan and Belgium. We report 7 cases from four families in Andalusia, identified during the quantification of Hb A1c.

In all cases, both by capillary electrophoresis and ion exchange HPLC (II-HPLC), HBX appeared faster than HbA. By reverse phase HPLC eluted globin chain slower than αA, αT. Sequencing of gene α2 shown the substitution (CGT>TGT) in exon 3 CD141(Arg>Cys)→Hb Nunobiki.

This hemoglobin has increased affinity for oxygen but was not detected by polycythemia due to the low percentage of variant since it is a chain, but because it showed during the determination of Hb A1c. This fact confirms that the II-HPLC is the gold standard for identification of hemoglobinopathies.

The quantification of Hb A1c, by HPLC can identify structural Hb variants that otherwise would not have been detected and are clinically silent but may help clarify the evolution, adaptation and distribution of hemoglobin to the medium.

Keywords: Genetic disorders; Hb nunobiki

Introduction

Common hemoglobinopathies are those genetic alterations of the hemoglobin (Hb) whose prevalence rates exceed 1% of the population of any given region of the world. HbS, Hb C, Hb E, α thalassemia and β thalassemia are the most frequent monogenic disorders in the world and are an important public health issue.

These common hemoglobinopathies seem to appear as an adaptation to the environment. Their populations of origin were subject to positive selection, because heterozygotes got protected against Plasmodium falciparum [1] malaria.

These genes are expanded by genetic drift mechanisms and driven by major events in human history. Thus, the development of agriculture and the subsequent appearance of the first human settlements contributed suitable conditions to consolidate malaria as a holoendemic disease, as well as the endemicity of the genes of these hemoglobinopathies [2].

The explorations of the coasts of Africa, the discovery of America in the 15th century and its subsequent colonization were significant driving forces behind slave trade to these regions. Over the following centuries, England and most other European nations (Spain, the Netherlands, Denmark, France…) set sail to the American colonies. The triangular trade of black slaves took off since then and so did the spreading of these pathological genes to new areas. Travelers to and from the East Indies also contributed to spread these genes [3].

In fact, the first clinically recognized hemoglobinopathy was Hb S, discovered in Chicago by James Herrick back in 1910 when he observed sickle-shaped red blood cells in the blood of a black Jamaican student with anemia [4]. However, there is a different type of hemoglobinopathies, silent in most cases, with very low prevalence rates that get reported in common hemoglobinopathies studies or in studies of glycosylated hemoglobin in diabetics patients. Most of these variants have been identified in isolation, some others across a limited number of families that, in some cases, are not geographically close to each other. These last cases are credited to de novo mutations or to instances on which the gene mutation has been carried involuntarily [5].

A good example of this kind of distribution and spreading is Hb Nunobiki. The first case of Hb Nunobiki was reported in 1985 on a Japanese male [6]. Eleven years later, a second case was reported on a Belgian woman with no Japanese background, which would suggest an instance of de novo mutation [7]. In this paper, we are reporting 7 cases of Hb Nunobiki found in four families located in the same region in Spain (Andalusia).
Material and Methods

Between January 2011 and April 2012 we detected an interference in the measurements of Hb A1c using a VARIANT™ II TURBO Hemoglobin Testing System for HbA1c kit 2.0 (Bio-Rad) in 7 diabetic patients (4 women and 3 men), from four families, living in Andalusia (Spain).

Hematological data were obtained in a hematological counter (Coulter LH750 Analyzer; Beckman Coulter, Brea, CA, USA). Hb A2 and Hb F values were quantified by ion exchange high performance liquid chromatography (HPLC) (VARIANT™, Bio-Rad Laboratories, Hercules, CA, USA). Hemoglobin (Hb) was studied using capillary electrophoresis (MiniCap Haemoglobin (E); Sebia, Evru Cedex, France) as well as ion exchange HPLC; finally, the globin chains were separated by reversed phase HPLC using a Vydac large pore C4 column (The Separations Group, Hesperia, CA, USA) and a linear gradient from 47 to 100.0% of phase B in 80 min. at a flow-rate of 0.8 mL/min [8].

Genomic DNA was extracted from peripheral blood leukocytes using an automated method (Bio-Robot® EZ1 (Qiagen GmbH, Hilden, Germany)). Molecular characterization was undertaken using automatic sequencing in an ABI PRISM™ 3100 Genetic Analyzer Sequencer (Applied Biosystems, Foster City, CA, USA). The α2 gene was specifically amplified with P1A (5’-AGC GCC GCC CGG CCG GGC GT-3’) and C3R [5’-CCA TTG TTG GCA CAT TCC GG-3’ primers, specifically amplified with P1A (5’-AGC GCC GCC CGG CCG GGC GT-3’) and C3R [5’-CCA TTG TTG GCA CAT TCC GG-3’ primers, specific to the 3’UTR (3’ untranslated region) of the α2-globin gene]. The product of the amplification was sequenced with the commercial ABI PRISM™ BigDye® Kit V1.1 Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA). The primers used for the sequencing were P1A, PB (5’-CCC GCC CGG ACC CAC A-3’) and PIC (5’-AGA TGG CCC CCT CTC AG-3’). α-thalassemia was ruled out by α-globin StripAssay (ViennaLab Diagnostic GmbH, Vienna, Austria).

Results

Hematological data for the 7 patients are listed in Table 1. None of the patients carried a α-globin gene triplication, nor were any of the most common nondeletional or point mutation α-thal determinants present. There were not microcytosis or hypochromia.

In all patients a fast-moving abnormal hemoglobin was detected both capillary electrophoresis and ion exchange HPLC (Hb X; Hb A and Hb A) (Figure 1a,b). By reversed phase HPLC a slower moving αx chain (10-13% of the total α globin chain) (Figure 1c).

The α2 gene was amplified by PCR and 947 bp of α2 gene were sequenced from 44 bp upstream of the DNA Cap site to 157 bp downstream of the termination site including the three exons and two introns. Sequence analysis showed a single base substitution (C>T) at codon 141 of exon 3. This alters the normally encoded arginine to cysteine (CGT>TGT), which is identified as Hb Nunobiki (Figure 2).

Discussion

Hemoglobinopathy distribution does not follow specific patterns, and Hb Nunobiki is testament to this. The first case reported in Japan, in 1985, probably was a de novo mutation. In the second reported case, the Belgian patient had no known relatives of Japanese descent, and therefore, this Hb would likely be the result of an independent mutation [6,7]. The seven cases reported in Spain were located in the same region (Andalusia), and could all have their origin on the same gene mutation whose involuntary dispersion might be related to a specific event occurred at some point in the distant past. During the 17th century, trade routes to and from the West and East Indies were led by the Spanish Crown, and Seville was the most important port and the place where people from every nation came to do business [9]. Between 1613 and 1620, Samurai Hasekura Rokuemon Tsunenaga led a diplomatic mission to Europe, crossing the Pacific and Atlantic oceans. They set foot in Europe in 1614 navigating the Guadalquivir River to Seville. Their mission lasted two years and, in 1618, the Japanese embassy returned to its country using the same port. But some members of the diplomatic mission decided to stay in Andalusia, more specifically in the province of Seville [10]. All the Hb Nunobiki cases reported were found in this very province. The incidence is too high for all seven to be considered de novo mutations, so we need to consider the origin of these cases to be on a gene inherited by diffusion or to be of different genetic origin.

Hb Nunobiki is a variant whose oxygen affinity is increased because the affected amino acid is residue 141 (Arg>Cys) and the COOH-terminal arginines of the alpha chains play a major role in normal human hemoglobin (HbA), both by electrostatic interactions which constrain deoxygenated hemoglobin in a low affinity quaternary conformation and by involvement in oxygen-linked anion binding known to give rise to a large part of the alkaline Bohr effect [6]. In spite of this, the cases reported by clinical literature display hematologic parameters that are on the upper limit of normal without pathological implications [6,7]. In this work, we have not been able to perform the functional study of hemoglobin by P50 determination, but the same behavior has been observed in our patients, whose Hb Nunobiki was detected because it appeared as an interference during Hb A1c determination (not due to the presence of polyglobulia or abnormal hematological parameters), which reveals that clinically silent hemoglobins can be found while quantifying glycosylated hemoglobin by off-Exchange HPLC. This can be relevant to prevent false Hb A1c values, on the one hand, and for genetic prophylaxis purposes, on the other hand. This confirms that the ion-exchange HPLC is the gold standard for the identification of hemoglobinopathies [11,12].

Erythrocritosis remains at minimum levels probably due to the fact that, being this an α chain variant, Hb Nunobiki relative weight on total hemoglobin is limited, although it can vary depending on the quantification technique applied. Ion-exchange HPLC, for instance, yields higher Hb Nunobiki percentage values (25.1-18.5%), since it is eluted together with Hb A, while capillary electrophoresis fails to detect Hb A and quantifies Hb Nunobiki only (14.1-10%).

Table 1: Hematological data.

|             | IA1 F | IA1 F | IA2 F | IB1 F | IC1 M | ID1 F | IE1 M |
|-------------|-------|-------|-------|-------|-------|-------|-------|
| RBC (10^12/L) | 4.8   | 5.1   | 4.7   | 5.2   | 4.4   | 4.9   | 5.7   |
| Hb (g/dL)    | 14.2  | 15.4  | 14.1  | 15.1  | 15.3  | 15.5  | 16.6  |
| PCV (L/L)    | 0.42  | 0.45  | 0.44  | 0.46  | 0.46  | 0.48  | 0.53  |
| MCV (fL)     | 87.8  | 86.5  | 92.6  | 88.0  | 105.9 | 97.5  | 91.6  |
| MCH (pg)     | 29.5  | 30.1  | 29.5  | 28.8  | 35.0  | 31.6  | 28.9  |
| MCHC (g/dL)  | 33.6  | 34.0  | 31.9  | 32.8  | 33.0  | 32.4  | 31.6  |
| RDW (%)      | 14    | 13.4  | 12.9  | 13.7  | 15.5  | 13.9  | 15    |
| Hb F (%)     | 1.2   | 0.9   | 1.2   | 1.0   | 1.0   | 0.6   | 1.6   |
| Hb A (%)     | 1.8   | 2.0   | 2.0   | 2.0   | 1.8   | 2.5   | 7.2   |
| HbX+HbA (%)  | 25.1  | 22.4  | 22.1  | 19.7  | 18.5  | 18.6  | 18.6  |
| HbX (%)      | 13.6  | 10.3  | 12.9  | 10.4  | 14.1  | 10    | 12.7  |

F: Female; M: Male
Figure 1: Different chromatograms, A: ion exchange HPLC (Variant). B: capillary electrophoresis. C: reversed phase HPLC.

Figure 2: Direct automated sequencing of exon 3 of α gene showing Hb Nunobiki [codon 141 (CGT>TGT) Arg→Cys].
Five additional variants have been reported at CD 141(HC3) of the α chain: J-Camagüey [Arg>Gly; HBA2:c.424C>G]; Singapore [Arg>Pro; HBA2:c.425G>C]; Cubujuqui [Arg>Ser; HBA2:c.424C>A]; Legnano [Arg>Leu; HBA2:c.425G>T] and Suresnes [Arg>His; HBA2:c.425G>A] [13-17]. No hemoglobin functional study was conducted for the first two variants (J-Camagüey, Singapore), while the other three are high oxygen affinity variants, like Hb Nunobiki. All five variants display no hematological abnormality in heterozygous state and, consequently, the percentage of abnormal hemoglobin is 20-25%. The high oxygen affinity might be explained by a similar mechanism to that of the Hb Nunobiki.

Unlike the Histidine 146 of the globin β chain, the Arginine 141 of the globin α chain does not increase the Bohr effect, even though they are equivalent positions. The Arg α 141 side chains are squeezed out aided by a weakening of the Tyr 140 hydrogen bond as with the β chain, and the bond 141 Arg α1-127Lys 2 is broken.

In Hb Nunobiki, the replacement of the last amino acid of the outer surface of the α chain C-terminal region terminal -Arginine (basic) with Cysteine (ambivalent), implies the modification of its electrical charge, which contributes ease of detection by electrophoresis of any kind.

Thanks to new HPLC programs, it is possible to identify hemoglobin structural variants that would not be detected otherwise while quantifying Hb A1c. Although clinically silent, these variants can help clarify the evolution, distribution and adaptation of hemoglobin to the environment.

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