Clinical report

Association between hemoglobin Groene Hart and hemoglobin J-Paris-I: First case in Spain

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ABSTRACT

Background and objective: Thalassemias are the most frequent monogenic disorder around the world. α-Thalassemias are due to a deficiency of synthesis in the alpha-globin chain of the hemoglobin (Hb). Hb Groene Hart is a hyperunstable variant. In this work, we have studied 24 cases affected by Hb Groene Hart, one of them associated with Hb J-Paris-I.

Patients and methods: Twenty-four patients from 17 unrelated families were included in this study. The characterisation was done by sequencing.

Results: α; gene sequencing showed the mutation CCT→TCT (Pro→Ser) at codon 119 (Hb Groene Hart) in all patients. In one case, there was an association with Hb J-Paris-I.

Conclusions: In the Hb Groene Hart, the residue 119 of alpha-globin chain is affected. This amino acid has a key role in preserving the stability of alpha-globin chain. It is also remarkable the presence of this variant in both the immigrant and native population. Thus, the identification of Hb Groene Hart carriers should be considered in the screening of α-thalasemia in Spain, as it is done in Northern Africa.

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Asociación de la hemoglobina Groene Hart con la hemoglobina J-París-I: primer caso en España

RESUMEN

Fundamento y objetivo: Las talasemias son las enfermedades monogénicas más frecuentes a nivel mundial. Representan un grave problema sanitario en las regiones de mayor incidencia. Las α-talasemias se deben a un déficit de síntesis de las cadenas α de la hemoglobina (Hb). La Hb Groene Hart es una variante hiperinestable. En este trabajo se presentan 24 casos pertenecientes a 17 familias afectadas por la Hb Groene Hart, uno de ellos asociado con Hb J-Paris-I.

Pacientes y método: Veinticuatro pacientes de 17 familias no relacionadas fueron incluidos en este estudio. La caracterización se realizó mediante secuenciación.

Resultados: La secuenciación del gen α mostró la mutación CCT→TCT (Pro→Ser) en el codón 119 (Hb Groene Hart). En un paciente se asoció con la Hb J-Paris-I.

Conclusión: En la Hb Groene Hart se encuentra afectado un residuo clave para preservar la estabilidad de las cadenas α de globina. La presencia de esta variante es elevada en población española e inmigrante. La aparición de formas graves de la enfermedad podría ser evitada incorporando esta mutación al cribado de las mutaciones α-talasemia no delección.

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Introduction

Thalassaemias are the most common monogenic disorders around the world and they represent a serious health problem in areas with a higher incidence. α-Thalassaemias are caused by the reduced or absent synthesis of the chain α of hemoglobin (Hb) and are characterised by microcytic and hypochromic anaemia.\(^1\) Their main molecular mechanisms are major deletions. However, about 5–10% of α-thalassaemias are due to specific mutations (non-deletion α-thalassaemia), which lead to a transcription, translation or post-translation processing defect.\(^2\) The latter produces the so-called hyperunstable Hb, which are not detected by most of the electrophoretic or chromatographic methods.

The Hb Groene Hart, also called Hb Bernalda, is a hyperunstable variant which early precipitates in erythrocyte precursors and is characterised by the specific mutation CCT→TCT at codon 119 of gene α\(_1\), thus causing proline (Pro) to serine (Ser) in the H2 position of the peptide chain.\(^3\)

The variant was first described in 2 members of the same Moroccan family, who showed the phenotype of mild α thalassaemia with microcytosis or hypochromia.\(^3\)

It was also found in homozygosis in a 32-year old Moroccan woman with a phenotype of α-thalassaemia\(^a\) and has been associated with the α3.7 deletion in a Tunisian girl who had 3% of Hb Bart when born.\(^5\)

In this work, we have studied 24 cases of 17 families affected by Hb Groene Hart, one of which was associated with Hb J-Paris-I.\(^6\)\(^11\)

Clinical observation or methods

From January 2009 to April 2013, we studied 998 patients with α-thalassaemia who were referred to our centre because they had microcytosis without iron deficiency and because β-thalassaemia and β-thalassaemia had been previously ruled out. All of the studied patients provided their informed consent.

Hematological data were obtained through an hematological counter (Coulter® LH750 Analyser; Beckman Coulter, Brea, CA, U.S.A.). The quantification of Hbs A\(_2\) and F was conducted by means of a high-performance liquid chromatography (HPLC) of ion exchange (VARIANT™, Hemel Hempstead, United Kingdom). The Hbs were studied using capillary electrophoresis (Sebia® hispania S.A., Barcelona, Spain) and HPLC of ion exchange (VARIANT™, Hemel Hempstead, United Kingdom) and globin-chains using reversed phase HPLC (Shimadzu, Kyoto, Japan).

The molecular study required the automatic extraction of genomic DNA (BioRobot® EZ1; Qiagen, Hilden, Germany). The most common mutations were ruled out through the α-globin StripAssay® (ViennaLab Diagnostica GmbH, Vienna, Austria), and the molecular characterisation was conducted through specific automatic sequencing carried out in a sequencer ABI PRISM® 3100 Genetic Analyser (Applied Biosystems, Foster City, CA, U.S.A.), using the commercial kit ABI PRISM® BigDye® Terminator V1.1 Cycle Sequencing Ready Reaction Kit (PE Applied BioSystems, Foster City, CA, U.S.A.).\(^12\)

Results

Out of the 998 studied patients, 875 presented major deletions in globin clusters and 126 showed specific mutations (non-deletion α-thalassaemia). Within this last group with non-deletion α-thalassaemia, 24 patients (from 17 unrelated families, 6 of which were Maghrebi families) showed the mutation CCT→TCT (Pro→Ser) at codon 119 (Hb Groene Hart). Therefore, in our series of patients, the Hb Groene Hart accounts for 19% of non-deletion α-thalassaemia.

The hematimetric parameters, Hb A\(_2\), Hb F and the α-cluster genotype are included in Table 1.

The Hb ranges from 8 to 16 g/dL. The mean corpuscular volume (MCV) ranges from 70.2 to 85.6 fl and the mean corpuscular Hb (MCH) ranges from 23 to 27.6 pg. They all showed an Hb A\(_2\) within normal limits (2.2–3.5%), with normal Hb F and ferritin.

No anomalous Hbs were separated using capillary electrophoresis or HPLC of ion exchange.

Selective sequencing of gene α\(_1\) showed Hb Groene Hart. Besides, a patient also showed the mutation GCC→GAC (Ala→Asp) at codon 12 of the gene α\(_2\) (Hb J-Paris-I). All mutations were found in heterozygosis (Figs. 1 and 2).

Discussion

Hyperunstable Hb carriers have a thalassaemic syndrome due to the post-translational precipitation of anomalous chains. This kind of Hb variant accounts for 9.5% of structural hemoglobinopathies, and it is necessary to take them into account since it is complicated to detect them through electrophoretic techniques and HPLC of ion exchange.\(^13\)

In the Hb Groene Hart [CD119(H2) Pro→Ser α\(_1\)], the residue 119 of the ochain is affected. This residue has a key role in interacting with the alpha hemoglobin stabilising protein (AHSP)\(^\alpha\), and, thus, in preserving the stability of the entire globin chain. The AHSP is a chaperone which binds to the globin chains to prevent their precipitation, since the latter would damage the erythrocyte membrane. In the case of Hb Groene Hart, their hyperunstability is due to the affinity reduction caused by AHSP.\(^14\)\(^15\)

This mechanism has been suggested for other variants whose modified residue is located in G or H helices, since these interact with AHSP. The variants that would behave like this are, among others, Hb Bronovo [CD103(G10)His→Leu α\(_2\)], Hb Suan Dok [CD109(G16)Leu→Arg], Hb Foggia [CD117(GHS)Phe→Ser] and Hb Utrecht [CD129(H12)Leu→Pro α\(_1\)].\(^16\)

However, in Hb Diamant [CD119(H2) Pro→Leu α\(_1\)], where the affected residue is the same as in Hb Groene Hart, heterozygotes show no thalassaemic phenotype.\(^16\) Though in Hb Diamant the substitution of proline for leucine does not change the polarity, in Hb Groene Hart the proline residue is carried away by a polar amino acid (Ser). This probably results in a lower distortion for Hb Diamant interactions. In fact, in vivo, the stability of the complex formed by AHSP and the α\(_1\)Hb Diamant chain is higher than the stability present in the normal chain, due to the higher apolarity shown by Leu over Pro.\(^14\)\(^17\)

However, several other Hbs whose affected residue is not located in G or H helices show instability similar to that of Hb Groene Hart, which evidences the importance of said areas in the interaction with chaperone AHSP.\(^16\)

Without taking into account the 2-year old boy with iron-deficiency anaemia, the mean value of Hb was 13.4 g/dL; it is only worth outlining the value of 16 g/dL observed in 2 patients which was related to smoking. They all showed hypochromia with a mean MCH of 25.8 pg, except for the case associated with Hb J-Paris-I (27.6 pg), which demonstrates that the most appropriate parameter for the diagnosis of α-thalassaemia is not MCV but MCH, provided that ≤27 pg. The MCV ranged from 71.3 to 85.6 fl, and the lowest values corresponded to children. In general, all the values were slightly above those found in mutations whose result is hyperunstable hemoglobinopathy but where the affected gene is α\(_2\), as in the case of Hb Agrino [CD29(B10)Leu→Pro; α\(_2\)].\(^18\) However, this fact could be explained by the lower expression of the gene α\(_1\) (where the mutation responsible for Hb Groene Hart is located) in relation to the gene α\(_2\).
### Table 1

Hematological parameters and genotype of the study subjects.

<table>
<thead>
<tr>
<th>Family</th>
<th>Origin</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Patient</th>
<th>Hb (g/dL)</th>
<th>MCV (FL)</th>
<th>MCH (pg)</th>
<th>Hb A2 (%)</th>
<th>Hb F (%)</th>
<th>Reticulocytes (100×)</th>
<th>Genotype</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>Madrid</td>
<td>55</td>
<td>M</td>
<td>II</td>
<td>16b</td>
<td>82.3</td>
<td>27.6</td>
<td>2.2</td>
<td>2</td>
<td>0.8</td>
<td>ααt/A1Pm</td>
</tr>
<tr>
<td>B</td>
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<td>35</td>
<td>F</td>
<td>II</td>
<td>11.8</td>
<td>77.3</td>
<td>26.3</td>
<td>2.5</td>
<td>0.8</td>
<td>–</td>
<td>ααt/Aαt</td>
</tr>
<tr>
<td>C</td>
<td>Gipuzkoa</td>
<td>–</td>
<td>M</td>
<td>II</td>
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<td>77.9</td>
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<td>2.9</td>
<td>0.5</td>
<td>3.1</td>
<td>ααt/Aαt</td>
</tr>
<tr>
<td>D</td>
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<td>F</td>
<td>II</td>
<td>13.1</td>
<td>78.5</td>
<td>26</td>
<td>2.9</td>
<td>0</td>
<td>0.19</td>
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</tr>
<tr>
<td>E*</td>
<td>Navarre</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>13.9</td>
<td>77</td>
<td>24.5</td>
<td>3.4</td>
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<td>ααt/Aαt</td>
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<td>–</td>
<td>F</td>
<td>II</td>
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<td>82.4</td>
<td>24.5</td>
<td>2.9</td>
<td>0.6</td>
<td>2.2</td>
<td>ααt/Aαt</td>
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<tr>
<td>G†</td>
<td>Navarre</td>
<td>37</td>
<td>M</td>
<td>II</td>
<td>13.9</td>
<td>79.5</td>
<td>26</td>
<td>3.2</td>
<td>0.4</td>
<td>1.75</td>
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<tr>
<td>H*</td>
<td>Lanzarote</td>
<td>–</td>
<td>–</td>
<td>II</td>
<td>14.2</td>
<td>71.3</td>
<td>23</td>
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<td>0.2</td>
<td>1.85</td>
<td>ααt/Aαt</td>
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<td>–</td>
<td>M</td>
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<td>81.5</td>
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</tr>
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<td>K</td>
<td>Madrid</td>
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<td>F</td>
<td>II</td>
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<td>L</td>
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<td>F</td>
<td>I2</td>
<td>12.9</td>
<td>71.9</td>
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<td>ααt/Aαt</td>
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<td>M*</td>
<td>Gran Canaria</td>
<td>–</td>
<td>M</td>
<td>II</td>
<td>14</td>
<td>84.4</td>
<td>27</td>
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<td>0.2</td>
<td>0.99</td>
<td>ααt/Aαt</td>
</tr>
<tr>
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<td>Guadalajara</td>
<td>7</td>
<td>–</td>
<td>II</td>
<td>12.3</td>
<td>76.4</td>
<td>25.4</td>
<td>3</td>
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<td>0.32</td>
<td>ααt/Aαt</td>
</tr>
<tr>
<td>N*</td>
<td>Guadalajara</td>
<td>5</td>
<td>–</td>
<td>I2</td>
<td>12.3</td>
<td>76.7</td>
<td>25.1</td>
<td>3</td>
<td>0.4</td>
<td>0.41</td>
<td>ααt/Aαt</td>
</tr>
<tr>
<td>O</td>
<td>Toledo</td>
<td>43</td>
<td>F</td>
<td>II</td>
<td>13.7</td>
<td>85.6</td>
<td>26.4</td>
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<td>0.37</td>
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<tr>
<td>P</td>
<td>Toledo</td>
<td>34</td>
<td>F</td>
<td>I2</td>
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<td>84.7</td>
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<td>Q</td>
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<td>I2</td>
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<td>84.4</td>
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<td>0.2</td>
<td>1.08</td>
<td>ααt/Aαt</td>
</tr>
</tbody>
</table>

M: male patient; Hb: hemoglobin; MCH: mean corpuscular hemoglobin; F: female patient; MCV: mean corpuscular volume; ααt: Groene Hart; α1Pm: J-Paris-I.

* Maghrebi families.
† Smoker.
† Iron-deficiency anemia.

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**Fig. 1.** Direct sequencing: hemoglobin Groene Hart or Bernalda (α1 119(H2) Pro→Ser): CCT→TCT.

**Fig. 2.** Direct sequencing: hemoglobin J-Paris-I (α2 12(A10) Ala→Asp): GCC→GAC.
In our study, we found no cases of homozygotes or double heterozygotes with a deletion in the other allele (α+ deletion thalassemia), though these have been observed in other studies. No patient showed Hb H disease, since this occurs when the Hb Groene Hart is associated with a deletion causing an α+ thalassemia deletion in the other allele.

The study observed the first case of Hb Groene Hart associated with Hb J-Paris [CD12(A10) Alα→Asp; α2]. No differences were observed regarding the rest of the patients. The Hb J-Paris is a fast variant which was first found in a Spanish female patient and was later on observed in Portugal, Iran, Yugoslavia and Italy. It is clinically silent and shows no hematological changes, which would confirm that its association with other variants does not worsen the symptoms.

In this study, the Hb Groene Hart was found in both the Spanish population and the immigrant population coming from the Maghreb, where it was first described and is relatively common. Given the geographical and historical relationship between the 2 populations, it is possible that the mutation had the same origin.

During the Middle Ages, between 711 and 1492, the Iberian Peninsula formed part of the so-called al-Andalus, which was under the Muslim control of the North-African Omeya Caliphathe. In 1492, the Catholic Kings put an end to the Islamic control over the Peninsula, though most of the Muslim population decided to adopt the new customs and remain in their lands. Eight centuries of Muslim control may have provided enough time for there to occur a miscegenation process which would make it possible for this variant to be currently present amongst the native population. This idea is reinforced by the fact that all the Spanish patients come from the southern area of the Peninsula (Table 1), except for a patient who came from the Basque Country but had Extremaduran origins. Therefore, the populations that had been in contact with Muslims the longest would be those that currently have Hb Groene Hart.

Both contributions (native and immigrant) have an Hb Groene Hart frequency of almost 20% of the non-deletion α-thalassaemia cases in our country. Such percentage is relatively high compared to other less common variants. Therefore, the molecular identification of the carrier of the mutation responsible for Hb Groene Hart should be taken into account when analysing non-deletion α-thalassaemia cases within the framework of microcytosis screening programmes, as it occurs in Northern Africa, so as to try to prevent, through genetic advice, the onset of severe forms of the disease.

Conflict of interest

The authors declare that there are no conflicts of interest.

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