

# Double heterozygosity for hemoglobin C and beta thalassemia dominant: A rare case of thalassemia intermedia

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## Abstract

Beta thalassemia dominant results from mutations in the  $\beta$  globin chain gene resulting in the production of elongated, highly unstable beta globin chains. Several such mutations have been described and in a heterozygous state they may confer a phenotype more severe than that of  $\beta$  thalassemia trait and lead to a clinical syndrome of thalassemia intermedia and its associated complications such as extramedullary hemopoiesis, bone disease, endocrinopathies and iron overload even in the absence of transfusion. In this report we present a case of double heterozygosity for HbC and  $\beta$  thalassemia dominant leading to a series of complications that were treated successfully once the correct diagnosis was made.

## Introduction

Beta thalassemia is a genetic disorder, one of the most common worldwide, characterized by decreased synthesis of  $\beta$  globin chains. Three types have been defined, based on the degree of reduced beta-globin chain synthesis and clinical phenotype: major, intermedia and minor (heterozygote carrier state). However, currently, based on the transfusion requirements, thalassemia syndromes can be classified into two main groups: transfusion dependent thalassaemias (TDTs) and non-transfusion dependent thalassaemias (NTDTs). The TDTs require regular blood transfusion as without adequate transfusion support, patients would suffer several complications and a short life span.  $\beta$ -thalassemia major belongs, among others, to this category. The group of NTDT patients includes  $\beta$ -thalassemia intermedia and patients with thalassemia trait.

In this case report we discuss a rare case of double heterozygosity for Hemoglobin C (HbC) and a beta globin mutation resulting in a beta-thalassemia intermedia phenotype.

## Case Report

A 45-year-old gentleman was referred to our hemoglobinopathy clinic by his general practitioner (GP) for management of his *sickle cell disease*. In the past, he had developed 2 episodes of spinal cord compression requiring decompression surgery (in 2003 and 2013) secondary to extramedullary hematopoietic pseudotumors in the pre-sacral area. Extramedullary hematopoiesis (EMH) is the production of red blood cells outside of the bone marrow and may occur as a consequence of hematological disorders such as myelofibrosis and chronic anemia in thalassemia.<sup>1</sup> However, he was lost to any hematology follow-up. In addition, he had been diagnosed in primary care as suffering of sickle cell disease and he was managed, until his early 40s, with analgesia for *painful crises* in various emergency departments. Clinically, he was complaining of long-standing generalized bone pains and constitutional symptoms such as weight loss, lethargy and night sweats. There was no demonstrable neurological deficit.

Review of his clinical history and investigations revealed a discrepancy between diagnosis and clinical phenotype. His hemoglobin (Hb) was 86 g/L with normal red cell indices and his ferritin was raised at 948  $\mu$ g/L. The peripheral blood smear showed aniso-poikilocytosis with occasional *boat cells* and crystal-containing erythrocytes. High-performance liquid chromatography (HPLC) showed no Hemoglobin A (HbA), Hemoglobin C (HbC) of 78% and Hemoglobin F (HbF) of 14%; no Hemoglobin S (HbS) was detected (Figure 1). This pattern excluded sickle cell disease but was insufficient to explain his significant anaemia and his clinical presentations between 2003 and 2013. He had a clinical picture more consistent with a diagnosis of thalassemia intermedia. A definitive diagnosis was made upon DNA analysis showing double heterozygosity for HbC and a beta globin gene mutation in codon 177 (-C) [HBB: c.354delC which results in the production of an elongated highly unstable beta globin, which acts in a dominant fashion, leading to a thalassemia intermedia phenotype. Alpha chain analysis was normal ( $\alpha\alpha/\alpha\alpha$ ).

Further investigations showed complications of untreated thalassemia intermedia. A DEXA scan (spine and both femora) showed osteoporosis (T score -2.6), associated with a high risk of spontaneous fractures. Liver MRI (ferriscan) showed moderate hepatic siderosis with a liver iron concentration (LIC) of 8.2 mg/g of dry tissue

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(normal 0.17-1.8) but a cardiac T2\* was normal with a value of 43 ms. MRI spine showed the presence of a sacral mass secondary to EMH (Figure 2).

He was commenced on a *hyper-transfusion* program with an aim to maintain a pre-transfusion Hb level  $\geq$  110 g/L and hydroxycarbamide in order to reduce the paraspinous EMH, preventing further neurological complications. An added effect of hydroxycarbamide was to increase HbF levels and ameliorate his anaemia.<sup>2</sup> Indeed his HbF% increased from 14% in October 2015 to 24.5% in February 2017. To manage his iron overload,<sup>3</sup> the patient was started on the iron chelating agent (deferasirox).<sup>4</sup> Finally, he has commenced on Alendronic acid, 70 mg, once weekly to improve his osteoporosis and reduce spontaneous fracture risk. He is under regular follow-up by endocrinology. The combination of these treatments had a profound impact on the patient's general health; he remains mostly asymptomatic, his constitutional symptom resolved and has not had any further neurological complications.

## Discussion and Conclusions

Beta thalassemia is inherited in a recessive pattern when there is a quantitative reduction of  $\beta$  globin chains that are usually

structurally normal.<sup>5</sup> Gene expression at transcription or post-transcriptional level has been identified in  $\beta$  thalassemia, leading to a reduction in  $\beta$  globin in a variety of levels.<sup>6</sup> Synthesis of normal  $\alpha$  globin chains, from the unaffected  $\alpha$  globin gene, continues as normal, leading in accumulation of excess unmatched  $\alpha$  globin. Those  $\alpha$ -globin chains are not able to form tetramers and instead precipitate in the erythroid precursors forming inclusion bodies. They are responsible for the extensive intramedullary destruction of the erythroid precursors and the ineffective erythropoiesis described in beta thalassemia.

Dominant cases of thalassemia are also described in the literature regarding cases of thalassemia major or intermedia phenotype which are associated with homozygosity or compound heterozygosity for thalassemia mutations.<sup>7,8</sup> More than 250 mutations have already been reported that could potentially cause  $\beta$  thalassemia.<sup>9</sup> Around 1/6 of them, have been associated with the dominant form of  $\beta$  thalassemia.

This type of beta thalassemia has been described in various geographical areas and is not prevalent in malaria endemic places.<sup>10</sup> In dominantly inherited  $\beta$ -thalassemia, there is a functional deficiency of  $\beta$  globin. Precipitation of the  $\beta$ -chain variants along with the excess of  $\alpha$  chains, overload the proteolytic intracellular mechanisms increasing ineffective erythropoiesis. The large intra-erythroblastic inclusions, which are characteristic of this form of  $\beta$ -thalassemia, are composed of both  $\alpha$ - and  $\beta$ -globin chains.<sup>11</sup> On the other hand, the inclusion bodies in homozygous  $\beta$ -thalassemia consisted only of precipitated  $\alpha$  globin.

Behind that molecular mechanism, which favors instability, are various mechanisms like substitution of amino acids in the hydrophobic heme pocket displacing heme, leading to aggregation of the globin variant, disruption of secondary structure because of replacement of critical amino acids, substitution or deletion of amino acids involved in  $\alpha\beta$  dimer formation and elongation of subunits by a hydrophobic tail.

The dominant type mutations could include missense mutations, nonsense mutations or minor insertions or deletions.<sup>8</sup> Examples of a missense mutation causing  $\beta$ -thalassemia intermedia include Hb Terre Haute ( $\beta 106$  Leu  $\rightarrow$  Arg),<sup>12</sup> originally described as Hb Indianapolis ( $\beta 112$  Cys  $\rightarrow$  Arg),<sup>13</sup> Hb Chesterfield,<sup>14</sup> Hb Cagliari,<sup>15</sup> Hb Showa-Yakushiji,<sup>16</sup> Hb Durham NC/Brescia,<sup>17</sup> Hb Houston,<sup>18</sup> and more recently, and Hb Mont Saint Aignan.<sup>19</sup>

Heterozygosity for a stop codon in  $\beta 127$  (CAG TO TAG) has also been found to

cause thalassemia intermedia in an English woman.<sup>20</sup>

Hemoglobin Hakari is an example where the characteristic is the change of B13 position on the globin chain corresponding to codon 31 on the globin gene leading to formation of an unstable hemoglobin molecule with altered tertiary structure.<sup>21</sup>

Another mutation described in the same site where lysine is replaced by proline giving rise to haemoglobin Yokohama.<sup>22</sup>

Examples of deletion mutations are Hb Korea,<sup>23</sup> Hb Gunma,<sup>24</sup> and Hb Stara Zagora.<sup>25</sup>

Individuals who carry those mutations could present with intermedia  $\beta$  thalassemia or more severe types. The excess of  $\alpha$  globin chains could lead to ineffective erythro-

poiesis and patients could present with jaundice, splenomegaly and extramedullary hematopoiesis.<sup>26</sup>

DNA based diagnostics can readily detect and confirm these uncommon  $\beta$  globin gene mutations and help implement appropriate treatment planning and genetic counseling.<sup>27</sup>

Hemoglobin C is a structural variant of normal hemoglobin A (Hb A) caused by an amino acid substitution of lysine for glutamic acid at position six of the beta hemoglobin chain. There are various combinations of hemoglobin C like hemoglobin C trait, where hemoglobin C is found together with normal adult hemoglobin, sickle cell-hemoglobin C disease, in which hemoglobin C and hemoglobin S occur in approxi-

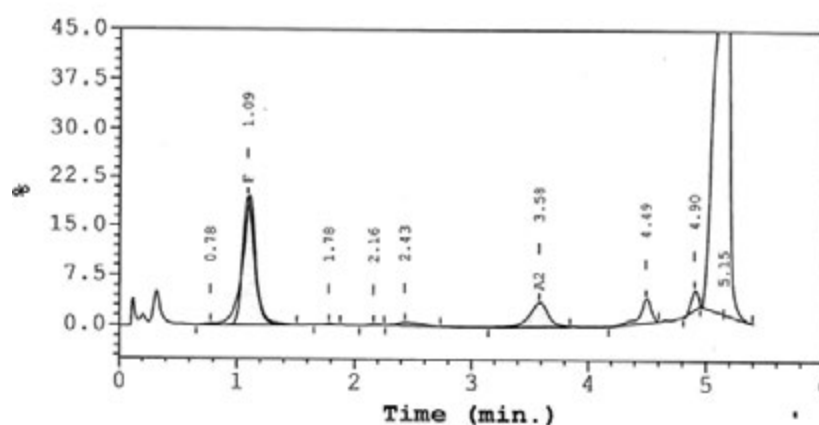


Figure 1. High performance liquid chromatography at presentation.



Figure 2. Sacral mass (arrow) due to extramedullary haemopoiesis.

mately equal amounts, HbC disease where virtually all the hemoglobin is of this abnormal variety and hemoglobin C-thalassemia in which the hemoglobin electrophoretic pattern resembles that of HbC disease, but in which the affected individual has a single genetic factor for hemoglobin C in addition to a gene for thalassemia.

The case that we describe has further interest as the patient was found to have a double heterozygosity for HbC and beta thalassemia dominant. In this case, co-inheritance with HbC led to the erroneous diagnosis of sickle cell disease (SCD) even though the patient had no HbS.  $\beta$  thalassemia dominant is a recognized cause for thalassemia intermedia, and the presence of HbC may have contributed to the severity of the phenotype.

DNA analysis revealed the presence of double heterozygosity for Hb C and the beta globin mutation Codon 177 (-C) [HBB: c.354delC] while  $\alpha$  chain analysis was normal. This produced an elongated highly unstable beta globin that act in a dominant fashion and explains the thalassemia intermedia phenotype. By reviewing the literature, we found out that there was no other case describing the specific heterozygosity.

The patient was also found to be heterozygous for the beta globin gene mutation Codon 6 (GAG>AAG) [HBB:C19G>A] which give rise to the hemoglobin variant HbC. This variant is benign in the carrier state and is not thought to significantly influence the effect of co-existing beta thalassemia mutations, although it can result in a reverse sickling disorder when inherited with the HbS sickle cell mutation.

The diagnosis of such case is not easy and could lead to erroneous conclusions like in our patient, who was initially diagnosed with sickle cell disease. It is important to take under consideration medical history a have a high index of clinical suspicion. Electrophoresis could help in the setting of the diagnosis which will be established by DNA analysis.

Unfortunately, the patient could not provide any information about his parent but DNA analysis performed on his son, revealed that he is also heterozygous for the same dominant mutation for beta thalassemia, however, he is not carrying Hb C. His phenotype so far is mild which indicates that possibly it is the presence of Hb C that contributes to the patient's severe clinical picture. However, given the type of mutation, the son remains under follow-up.

Treatment is mainly supportive constituted by regular transfusions and hydroxycarbamide, as a modifying agent to suppress extramedullary erythropoiesis. For patients

with high transfusion requirements, splenectomy may be considered, especially in the presence of significant splenomegaly. Furthermore, iron chelation should be considered in case of iron load along with treatment of osteoporosis. Stem cell transplantation is an option in case there is a matched related donor and all indications are met favoring benefit over risk.

In summary, double heterozygosity of Hb C with dominant thalassemia is an extremely rare event which produces extremely unstable hemoglobin. The result of it is significant impairment in erythropoiesis along with a thalassemia intermedia phenotype which needs proper management otherwise the results could be devastating for the patient.

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