**Differences in the erythropoiesis-hepcidin-iron store axis between hemoglobin H disease and β-thalassemia intermedia**

Congenital anemias due to ineffective erythropoiesis, such as sideroblastic anemia, congenital dyserythropoietic anemia and β-thalassemia intermedia (TI), are frequently associated with increased dietary iron absorption and progressive iron loading, and are therefore defined as “iron loading anemias”.

Finch introduced the concept of the “erythroid regulator” of iron balance to illustrate the physiological mechanism by which the erythroid marrow expansion combined with ineffective erythropoiesis induces a positive iron balance.

The identification of novel molecules that regulate iron metabolism has provided a deeper insight into the pathophysiology of iron loading anemias. In a mouse model of TI, increased iron absorption was found to be mediated by downregulation of hepcidin and upregulation of ferroportin. In patients with TI, an inverse relationship was observed between urinary hepcidin levels and both serum erythropoietin and soluble transferrin receptor (sTfR), taken as markers of erythropoietic activity. A number of studies investigated the hypothesis that release of the transforming growth factor β (TGF-β) superfamily members by the erythroblasts during the process of ineffective erythropoiesis might interfere with hepcidin production. The findings of these studies suggest that excess release of growth differentiation factor 15 (GFD15) and twisted gastrulation (TWSG1) may suppress hepcidin production dysregulating iron homeostasis in thalassemia syndromes. More recently, the hormone erythroferrone (ERFE), has been identified as a new erythroid regulator of hepcidin synthesis.  

β-thalassemia intermedia, hemoglobin E/β-thalassemia (mild and moderate forms), and hemoglobin H (HbH) disease are three clinically distinct forms of the so-called non-transfusion-dependent-thalassemias (NTDTs), a term used to label patients with hemoglobinopathies who do not require regular transfusion for survival. In patients with TI, the broad diversity of β-globin gene mutations, the co-inheritance of α-thalassemia, and the presence of genetic determinants associated with increased production of γ-globin chains in adult life are the main determinants for milder α/non-α-globin chain imbalance and transfusion independence. These factors are of particular relevance in Sardinia, where the most common type of TI as well as of β-thalassemia major is due to a nonsense mutation at codon 39 (c.118C>T) of the β-globin gene. HbH disease is the most severe non-fatal form of the α-thalassemia syndrome, mostly caused by molecular defects of the α-globin genes in which α-globin expression is decreased. HbH is a tetramer of β chains, which is unstable and causes a phenotype of mild to moderate chronic hemolytic anemia characterized by readily detectable HbH inclusion bodies in the peripheral blood cells.

TI and HbH disease may have similar degrees of anemia, but hemolysis rather than ineffective erythropoiesis (IE) is

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### Table 1. Characteristics of patients with β-thalassemia intermedia and hemoglobin H disease.

<table>
<thead>
<tr>
<th>Genotype (conventional name, HGVs genotype [n])</th>
<th>Sex M/F</th>
<th>Age, years</th>
<th>Hb, g/dL</th>
<th>MCV, fl*</th>
<th>MCH, pg*</th>
<th>Reticulocytes (%)</th>
<th>ANI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codon 39 (C&gt;T)/Codon 39 (C&gt;T)</td>
<td>19/19</td>
<td>38.5 [31.3-48.0]</td>
<td>8.3 [7.6-9.4]</td>
<td>80.0 [73.0-87.5]</td>
<td>24.4 [22.4-27.1]</td>
<td>2.5 [1.8-3.7]</td>
<td>81100 [50150-135900]</td>
</tr>
<tr>
<td>Delta beta 0/Codon 39(C&gt;T)</td>
<td>16/20</td>
<td>36.0 [20.0-50.0]</td>
<td>8.9 [8.4-9.2]</td>
<td>61.0 [58.5-65.6]</td>
<td>19.0 [18.4-19.7]</td>
<td>2.9 [2.4-3.8]</td>
<td>134200 [114000-163500]</td>
</tr>
<tr>
<td>Codon 39 (C&gt;T)/Codon 6(-A)</td>
<td>19/19</td>
<td>36.0 [20.0-50.0]</td>
<td>8.9 [8.4-9.2]</td>
<td>61.0 [58.5-65.6]</td>
<td>19.0 [18.4-19.7]</td>
<td>2.9 [2.4-3.8]</td>
<td>134200 [114000-163500]</td>
</tr>
<tr>
<td>Codon 39 (C&gt;T)/Hb SHELBY</td>
<td>19/19</td>
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<td>134200 [114000-163500]</td>
</tr>
</tbody>
</table>

Values are given as median [25th-75th percentile]. AN: absolute number; *significantly different between β-thalassemia intermedia and hemoglobin H disease (P<0.05). Mild phenotype, other phenotypes are mild or severe (Online Supplementary Table S1A). HbH. α2(2-T) (5 mF), c.[95+2_95+6delTGAGG] recognized by the restriction enzyme HphII. NcoI, but ACG to CGC, c.[2TPC], p.[Met Thr], recognized by the restriction enzyme NcoI.
the primary mechanism in HbH disease. Indeed, iron loading is much more common in TI than in HbH disease.

In this study, we investigated the relationship between erythroid activity, body iron status, and hepcidin levels in 38 subjects with TI and 36 with HbH disease, all followed at the Ospedale Regionale per le Micromie, Cagliari, Sardinia, Italy. Patients with TI and HbH disease had either never been transfused or had received only sporadic transfusions during infections, pregnancy or surgery (less than 10 blood units in total, 5 years or more before evaluation for the present study). Values are given as median (25th-75th percentile; d.w.: dry weight; sTfR: soluble transferrin receptor; LIC: liver iron concentration; md: median; n=18, of whom 13 have received blood transfusions, and 15 have been treated with desferrioxamine, although with suboptimal compliance; n=12; of whom 5 have received blood transfusions, and one has been treated with desferrioxamine; *Ferriscan R2 MRI technology (www.resonancehealth.com). Significance of difference between β-thalassemia intermedia and hemoglobin H disease: *P<0.05; **P<0.01; ***P<0.001.

Table 2. Iron and erythropoietic parameters of β-thalassemia intermedia and hemoglobin H disease.

<table>
<thead>
<tr>
<th></th>
<th>β-thalassemia intermedia</th>
<th>Hemoglobin H disease</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>38</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Erythropoietin, mU/ml (***)</td>
<td>111.5 [74.5-189.8]</td>
<td>29.0 [19.4-45.5]</td>
<td>2.6-34</td>
</tr>
<tr>
<td>sTfR, mg/L (***)</td>
<td>11.4 [8.1-13.4]</td>
<td>5.8 [5.0-7.0]</td>
<td>0.76-1.76</td>
</tr>
<tr>
<td>GDF15, pg/ml (***)</td>
<td>14268 [7990-22272]</td>
<td>1046 [758-1685]</td>
<td>82-1486</td>
</tr>
<tr>
<td>Serum hepcidin, nM (***)</td>
<td>0.25 [0.25-0.25]</td>
<td>2.4 [1.4-4.1]</td>
<td>Men &lt;0.5-14.7 (md, 4.3)</td>
</tr>
<tr>
<td>LIC, mg/g d.w.</td>
<td>7.0 [3.8-12.7]</td>
<td>5.1 [2.8-9.4]</td>
<td>0.17-1.8</td>
</tr>
<tr>
<td>Serum hepcidin/GDF15, μmol/hg (**)</td>
<td>0.02 [0.01-0.11]</td>
<td>2.4 [12.46]</td>
<td>Unknown</td>
</tr>
<tr>
<td>Serum hepcidin/ferritin, pmol/µg (***)</td>
<td>0.7 [0.5-1.0]</td>
<td>10.0 [7.30]</td>
<td>Men 2.9-87.9 (md, 26.7); Pre-menopausal women 3.0-167.3 (md, 35.7); Post-menopausal women 9.1-143.1 (md, 40.5)</td>
</tr>
<tr>
<td>Serum hepcidin/LIC, μmol x d.w./L (**)</td>
<td>0.04 [0.02-0.2]</td>
<td>0.6 [0.3-0.9]</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
(despite the iron chelation treatment) ferritin in this syndrome was significantly higher compared to HbH. LIC was also higher in TI, but this difference was not significant, probably due to treatment of most TI patients with desferrioxamine in combination with the relatively low number of patients for whom LIC was assessed (Table 2). Serum hepcidin was below the limit of detection in 30 subjects with TI and strongly reduced in the 8 others, while it was less than 0.5 nM only in one of the 36 patients with HbH disease; there was a significant difference in the proportion of hepcidin values below 0.50 nM between the two groups (P<0.001), while median values groups were 0.50 nM (range 0.50-0.50) and 2.4 nM (range 1.3-4.1), respectively (P=0.001).

A binary logistic regression model (Cox & Snell: R2=0.624) for the occurrence of unmeasurable values of hepcidin (≤0.50 nM vs. >0.50 nM) considering the disease type and severity, GDF15, erythropoietin, sTfR, serum ferritin and sex, showed three variables significantly associated with the occurrence of very low values of hepcidin: disease (Odds Ratio (OR)=46.0 for TI vs. HbH disease; P=0.004), levels of GDF15 (OR=1.5 per 1000 pg/mL increase; P=0.033) and levels of serum ferritin (OR=12.0 per 100 ng/mL decrease; P=0.040). These data illustrate that low values of hepcidin are strongly driven by erythropoietic factors and iron stores, and in a different manner for TI and HbH disease. These observations are consistent with GDF15 as the factor contributing most to inhibition of hepcidin levels, in agreement with its previously proposed causal role in the IE- hepcidin-ferritin cascade.4

In addition, both the hepcidin/ferritin ratio and the hepcidin/ LIC ratio were roughly 10-fold lower for TI than for HbH (Table 2 and Online Supplementary Figure S2). When compared to hepcidin/ferritin ratio of adults from a sample of the Dutch general population, patients with HbH have a median hepcidin/ferritin ratio that was substantially lower but still within the reference range (www.hepcidinanalysis.com).13 These observations of hepcidin levels that are inappropriately low for the body iron levels in TI, and to a lesser extent in HbH, corroborate the presence of a suppressive erythropoietic signal associated with IE that is strong in TI and only slightly increased in HbH.14,15 GDF15, erythropoietin and sTfR were significantly lower in the subjects with mild TI genotypes (n=7) than in the moderate and severe genotypes (n=31) (P<0.005), confirming the role of GDF15 as an IE response marker, whereas only sTfR was significantly lower in subjects with mild deletional HbH (n=39) compared to the more severe non-deletional HbH defects (n=7; P=0.003). It is interesting to note that, for these analyses, the number of subjects might be too low to allow definitive conclusions to be made.

This study demonstrates that the different extent of IE and chronic hemolysis in TI and HbH disease is well reflected by the diverse patterns of the GDF15-hepcidin-ferritin axis in the two populations, even at similar levels of anemia. In TI, the erythropoietic signal more strongly suppresses iron loading-induced signaling to hepcidin, and for this reason iron overload is more common in TI disease than in HbH disease. Larger studies are needed to clarify whether for both TI and HbH the various molecular arrangements in the respective β- and α-globin chains associated with different degrees of IE affect the above-mentioned axis and related tendency to develop iron overload.

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1 This work is dedicated to the memory and in honor of Renzo Galanello, who encouraged the study until the end of his career.

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References


