**INTRODUCTION**

More than 800 hemoglobin variants are known to this day. Most of them have no clinical relevance but a great number of hemoglobin variants are responsible for many symptoms, such as jaundice, leg ulcers, erythrocytosis, hematuria, cerebrovascular incidents, cyanosis, retinal and vitreous hemorrhages, priapism and many others.

It was a study of hemoglobin S by Pauling and co-workers that led to the concept of "molecular disease" and the subsequent development of the field of molecular biology. Studies of hemoglobin S also led to the elucidation of the double helix structure of DNA (Watson and Crick).

**STRUCTURE, FUNCTION AND NOMENCLATURE**

The various hemoglobins (Hb) present in normal red cells are tetramers of subunits called $\alpha$, $\beta$, $\gamma$, $\delta$:

- Hb A $\alpha_2 \beta_2$
- Hb F $\alpha_2 \gamma_2$ (fetal hemoglobin)
- Hb A$_2$ $\alpha_2 \delta_2$

The chains of 141 or 146 amino acid residues that constitute the primary structure of hemoglobins form seven or eight helical regions. The heme group, composed of a porphyrin ring with an iron atom in the centre, is tucked into a crevice between two helices. The oxygen enters the heme pocket and is bound between the distal histidine and the iron atom of heme.

The designation of the first known normal and abnormal hemoglobins were named according to the alphabet. But later on, based on the increasing number of new variants, it was recommended to name hemoglobin variants after the community, state, county or nationality of the index case, the hospital or medical centre in which the discoverer of the variant works (Huisman et al., 1998).

**INDICATION OF TESTING FOR HB VARIANTS**

Clinically significant Hb variants are usually first observed by routine hematological procedures. A low Hb level, microcytosis, hypochromia, reticulocytosis, bilirubinemia may suggest the possible presence of an $\alpha$- or $\beta$-thalassemia, or an unstable hemoglobin type. Furthermore, a high Hb level (erythrocytosis) together with appropriate clinical observations may suggest a hemoglobin variant with an increased oxygen affinity.

**BIОСIENTИA’S TESTING PROGRAMS**

For many years we performed, as a basic method, the electrophoresis on cellulose acetate. In the meantime, the chromatographic techniques, especially HPLC are so highly developed that we changed our testing program from electrophoretic to chromatographic methods. We therefore proceed as follows:

1. HPLC chromatography and evaluation of the chromatogram with the different peaks and for the results for HbA2, HbF and HbA. Most Hb variants can easily be detected by this method.

If there is an abnormal chromatogram, we continue with:

2. Isoelectric Focussing (IEF) to confirm or to get a more precise differentiation between different suspected variants.

In all cases of hemoglobin variants we routinely perform the:

3. Electrophoresis in acidic milieu: Citrate-Agar-Gel Electrophoresis (CAG) at pH 5.9–6.1 with reference hemoglobins.

Using these different steps and methods, usually more than 95% of all abnormal hemoglobins are detectable. For all additional structure analyses with different techniques (such as DNA analysis) we collaborate with colleagues from the Department of Biochemistry of the Hôpital Henri Mondor at Creteil near Paris, France.

**BIОСENTИA: NEW VARIANTS DETECTED**

During the last 20 years in performing hemoglobin studies we have detected in our lab in collaboration with our colleagues from Creteil 4 new variants:

1. Hb Ingelheim (also known as Hb Coimbra) $\beta_{99}$ (G1)Asp$\rightarrow$Glu (Wajcman, Behnken, 1991)
   Symptoms: Cyanosis with erythrocytosis

2. Hb Mainz, $\beta_{98}$ (FG5) Val$\rightarrow$Glu (Wajcman, Behnken, 1994)
   Unstable hemoglobin causing severe hemolytic anemia

3. Hb Frankfurt, $\alpha_{50}$ (CE8) His$\rightarrow$Gln (Wajcman, Behnken, 1998)
   Hemoglobin variant without any clinical relevance, found during HbA1c studies

4. Hb Ilmenau (C7) $\beta_{41}$ Phe$\rightarrow$Cys (Prehu, Behnken, 2002). Unstable hemoglobin with low oxygen affinity causing hemolytic anemia and cyanosis.
In our cooperation with many hospitals in the Middle East we have detected patients with Hb S, Hb D (Hb D Punjab and Hb D Iran), Hb E, Hb O Arab, and furthermore we were able to detect carriers of:

Hb E-Saskatoon, β22 Glu → Lys
Hb Fontainebleau, α21 Ala → Pro
Hb Handsworth, α18 Gly → Arg
Hb Okayama, β2 His → Gln
Hb Setif, α94 Asp → Tyr

**Further Hemoglobin Variants Detected in Our Laboratories:**

<table>
<thead>
<tr>
<th>Hb Name</th>
<th>Substitution</th>
<th>Hematology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb J-Wenchang-Wuming</td>
<td>α11 Lys → Gln</td>
<td>normal</td>
</tr>
<tr>
<td>Hb Shaare Zedek</td>
<td>α66 Lys → Glu</td>
<td>Anemia</td>
</tr>
<tr>
<td>Hb O Indonesia</td>
<td>α116 Glu → Lys</td>
<td>normal</td>
</tr>
<tr>
<td>Hb Sassari</td>
<td>α126 Asp → His</td>
<td>Polyglobuly</td>
</tr>
<tr>
<td>Hb Okayama (18 Families)</td>
<td>β2 His → Gln</td>
<td>normal</td>
</tr>
<tr>
<td>Hb Freiburg</td>
<td>β23 Val fehlt</td>
<td>Anemia</td>
</tr>
<tr>
<td>Hb J Auckland</td>
<td>β25 Gly → Arg</td>
<td>normal</td>
</tr>
<tr>
<td>Hb Rothschild</td>
<td>β37 Trp → Arg</td>
<td>Anemia</td>
</tr>
<tr>
<td>Hb Seattle</td>
<td>β70 Ala → Asp</td>
<td>Anemia</td>
</tr>
<tr>
<td>Hb Köln (5 Families)</td>
<td>β98 Val → Met</td>
<td>Anemia</td>
</tr>
<tr>
<td>Hb Camperdown</td>
<td>β104 Arg → Ser</td>
<td>Anemia</td>
</tr>
<tr>
<td>Hb Presbyterian</td>
<td>β108 Asn → Lys</td>
<td>normal</td>
</tr>
<tr>
<td>Hb Andrew Minneapolis</td>
<td>β144 Lys → Asn</td>
<td>Polyglobuly</td>
</tr>
</tbody>
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**References**