Hemolytic anemia due to the unstable hemoglobin Wien: manifestations and long-term course in the largest pedigree identified to date

Hemoglobinopathies are disorders of the hemoglobin (Hb) chain synthesis. They fall into two broad categories: quantitative disorders (thalassemia syndromes) and qualitative disorders (structural Hb variants). The structural Hb variants are due to altered amino acid sequence in the alpha (α)- or beta (β)-chains, affecting Hb properties, such as stability, solubility, and O_2-affinity. Resulting disorders vary from clinically silent conditions to severe illness.[1] Unstable Hb result from amino acid substitutions within the heme cavity or pocket of the α or β polypeptide chains. Among the around 1,200 hb variants, approximately 150 are unstable Hb. Instability of the Hb molecule may result in chronic hemolytic anemia characterized by Heinz body formation, jaundice and discoloration of the urine due to dipyrrolic pigment excretion. The inheritance mode is typically autosomal-dominant. Mutations resulting in unstable Hb are rare events, and with few exceptions (*e.g.* Hb Zürich, Hb Hasharon, and Hb Köln) affect single pedigrees. Most unstable Hb have normal electrophoretic mobility, which makes correct diagnosis a challenge.[2,3] Therefore, unstable Hb should be suspected in cases resembling a β-thalassemia trait, but associated with unusually brisk hemolysis and reticulocytosis.

The clinical condition due to the unstable Hb Wien was first reported in a young Austrian man, and his daughter in 1964.[4] The identified Hb represents a β-chain variant named Hb Wien by Kleihauer *et al.*[5] Subsequent laboratory studies characterized its structure and properties.[6-8]

The available knowledge about Hb Wien and the resulting disease was derived from the first identified patient and his daughter.[4-9] This paper summarizes the clinical and laboratory findings in all four patients of the largest known Hb Wien pedigree and adds information on the disease severity and long-term course.

The index case, an 11 months old girl, was referred for further workup of severe anemia (hemoglobin 7 g/dL) diagnosed in the setting of a febrile illness.

The child was born at term after an inconspicuous first pregnancy of the mother. During the first days of life, there was a mild hyperbilirubinemia, which did not require treatment. The child's growth and motor development until referral were age-appropriate. On physical examination, the child was in a good general condition; the skin and the eyes were not jaundiced, and the liver and the spleen were not palpable.

Family history: The father of the child (pedigree III.2), as well as the father´s mother (pedigree II.1) and grandfather (pedigree I.1) are known to have hemolytic anemia due to the unstable Hb Wien. The two cases of Hb Wien published by Braunsteiner *et al.*[4] are grandmother and great-grandfather of the referred child (Figure 1).

Table 1. Laboratory values at first referral of the index case (pedigree IV.2).

	Pedigree IV.2 (child)	Normal range for age	Pedigree III.2 (father)	Normal range (males)	Pedigree II.1 (grandmother)	Normal range (females)
CBC						
WBC [g/L]	14.0	6.0 -17.0	5.5	4.0 - 9.0	9.7	4.0 - 9.0
RBC [T/L]	4.1	3.50 - 5.00	3.84	4.20 - 5.50	6.28	4.00 - 5.20
Hemoglobin [g/dL]	8.5	10.0 - 15.0	8.7	14.0 - 17.0	11.8	12.0 - 16.0
Hematocrit [%]	27.2	37.0 - 49.0	29.5	40.0 - 50.0	39.7	38.0 - 48.0
MCV [fl]	66.3	74.0 - 102.0	76.8	80-0 - 98.0	63.2	80.0 - 98.0
MCH [pg]	20.7	23.0 - 31.0	22.7	28.0 - 33.0	18.8	28.0 - 33.0
MCHC [g/dl]	31.3	27.0 - 35.0	29.5	32.0 - 36.0	29.7	32.0 - 36.0
RDW-CV [%]	26.7	11.5 - 16.0	30.9	11.5 - 16.0	23.6	11.5 - 16.0
Reticulocytes [%]	3.4	0.5 - 1.5	11.46	0.5 - 1.5	5.36	0.5 - 1.5
Platelets [g/L]	622	140 - 440	309	150 - 400	408	150 - 400
Clinical chemistry						
AST [U/L]	36	10 - 35	32	10 - 50	21	10 - 35
ALT [U/L]	15	10 - 35	17	10 - 50	21	10 - 35
GGT [U/L]	15	<20	15	<60	14	<40
Bilirubin [g/dL]	0.92	<1.0	10.27	< 1.20	3.7	< 1.20
LDH [U/L]	287	120 - 300	329	135 - 225	216	135 - 214
Haptoglobin [g/L]	0.69	0.07 - 2.34	<0.10	0.30 - 2.00	<0.10	0.30 - 2.00
Serum iron [µg/dL]	37	35 -155	122	33 - 193	98	33 - 193
Transferrin [g/L]	2.81	2.00 - 3.60	2.02	2.00 - 3.60	2.24	2.00 - 3.60
Transferrin-Sat. [%]	9.3	16.0 - 45.0	42.8	16.0 - 45.0	31.0	16.0 - 45.0
Feritin [µg/L]	103	7 - 142	573	15 - 150	1081	15 - 150

CBC: complete blood count; WBC: white blood cell; RBC: red blood cell: MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW-CV: red blood cell distribution width; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transpeptidase; LDH: lactate dehydrogenase.

The laboratory values of the index case, the father and the grandmother performed at the first referral of the child are summarized in Table 1.

Hemoglobin studies were performed by alkaline agarose gel electrophoresis (run semi-automatically on a Sebia HYDRASYS 2SCAN) and high performance liquid chromatography (HPLC) (BIO-RAD VARIANT II Hemoglobin testing System in Beta-Thal-Mode). The results of the HPLC are presented in Table 2 and illustrated in the *Online Supplementary Figure S1*. The gel electrophoresis is illustrated in the *Online Supplementary Figure S2*.

Molecular testing for the expected mutation was performed by a simple cycle sequencing technology (dideoxy chain termination/cycle sequencing on ABI 3730XL). The heterozygous mutation c.391T>G was confirmed in the pedigree members IV.2, III.2, and II.1.

The index case (pedigree IV.2) a now 2.5 years old girl with an age-appropriate growth and development. Her average Hb is stable at 9.0 g/dL. Lower values have been observed only at initial referral and during a febrile infection lasting for a week (hemoglobin of 7.0 and 7.3 g/dL, respectively). Recent sonography revealed a slightly enlarged spleen (longitudinal dimension of 90 mm; normal range 33-71 mm), which is clinically still not palpable.

The father (pedigree III.2) is now 37 years old. His average Hb was always in the range of 8.5-9.5 g/dL. He experienced two hemolytic and one aplastic crisis during his childhood. Two transfusions of packed red cells were necessary to date (one for aplastic crisis and one for cholecystectomy). His bilirubin varies between 3.3 and 14.2 mg/dlL, which is partly due to genetically confirmed Gilbert syndrome. Symptomatic gallstones prompted cholecystectomy at the age of 20 years. His spleen is palpable at 4 cm below the costal margin. Recent magnetic resonance imaging (MRI) assessment ruled out myocardial and liver iron overload.

The grandmother (pedigree II.1) is now 60 years old. Her average Hb is in the range of 8.0-9.0 g/dL. She has received a total of six packed red cell transfusions (two for surgery and two each during the two pregnancies). She had one endoscopic retrograde cholangio-pancreatography for gall sludge but has had no cholecystectomy yet. Her spleen is palpable at 4-5 cm below the costal margin. MRI assessment for iron overload was deferred for lack of consent.

The great-grandfather (pedigree I.1) was splenectomized at the age of 16 years.[4] Afterwards, he was reported to have thrombocytosis and recurrent thromboembolic episodes[9] and died of an pulmonary embolic event at the age of 49 years.

Hb Wien is an unstable hemoglobin variant, caused by the amino acid substitution β130(H8) Tyr-Asp at the base of the heme pocket.[6] It causes mild to moderate microcytic, hypochromic anemia with elevated reticulocyte counts and biochemical signs of hemolysis (Table 1). A recently published case had similar features.[3] Inclusion bodies (Heinz bodies) are present in 1-2% of the red blood cells before splenectomy, but after spleen removal, they raise to 30-40%.

Potentially misleading Hb electrophoresis is one of the major challenges of correctly diagnosing unstable Hb.[3] Unstable Hb can be missed either due to migration properties indistinguishable from Hb A1 or due to their rapid degradation. In cationic HPLC, Hb Wien does not separate from Hb A0 (*Online Suppementary Figure S1*).

In alkaline agarose gel electrophoresis Hb Wien and Hb A1 overlap (*Online Suppementary Figure S2*), while in polyacrylamide disc electrophoresis Hb Wien migrates somewhat faster and can be clearly separated from Hb A1, as reported previously.[10] Previously reported quantitative separation revealed Hb A1 70.4%, Hb Wien 24.2%, Hb A2 2.5% and Hb F 2.9%.[5] Hb Wien quantification at 24% is most probably due to its instability. Further characteristics of Hb Wien are an almost normal methemoglobin formation, slightly higher susceptibility to spontaneous oxidation and heat instability at 50°C, as well as an increased osmotic fragility.[4,5,7]

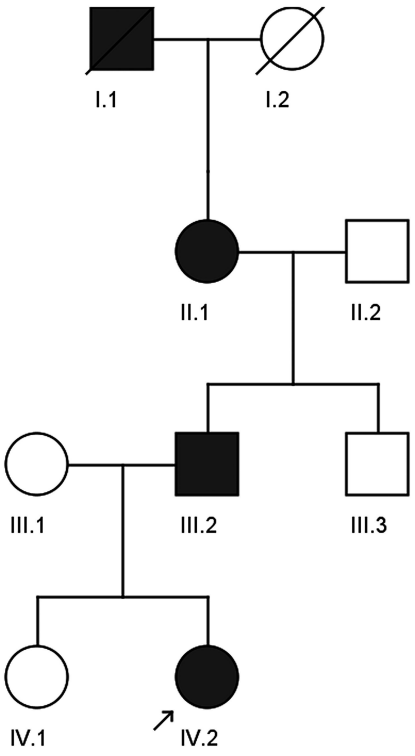

Figure 1. Pedigree of the largest family affected by hemoglobin Wien (Hb Wien).

Unlike β-thalassemia, Hb Wien is not associated with ineffective erythropoiesis.[7,9] Neither the bone marrow examination in I.1 and the nuclide scan findings in I.1 and II.1, nor the clinical follow-up of the patients have revealed signs of extramedullary erythropoiesis.[7,9]

The original report of the first patient (pedigree I.1) stated that the condition of the patient worsened at the age of 16 years, and repeated transfusions were necessary.[4] However, this report stays in contradiction to our observation of the other three family members, who have compensated hemolysis and relatively stable Hb levels.

Similarly to other chronic hemolytic anemias, Hb Wien is also associated with a risk for developing gallstones. This complication in patient III.2, who underwent cholecystectomy for symptomatic gallstones at the age of 20 years, was possibly accelerated by a coinheritance of Gilbert syndrome.

Patients with chronic hemolysis may be at risk for developing iron overload over time. The two members of the Hb Wien pedigree (II.1 and III.2), who have been followed for more than thirty years, still have a transferrin saturation within the normal range (Table 1). The lack of

Table 2. Hemoglobin high performance liquid chromatography at first referral of the index case (pedigree IV.2).

Peak name	Pedigree IV-1 (child)	Normal range for age	Pedigree III-2 (father)	Pedigree II-1 (grandmother)	Normal range adults
A1a window	3.0%		0.8%		
A1b window	0.7%		0.7%		
F window	16.0%	<15%	3.6%	4.6%	<1.0%
A1c window	4.8%		3.4%	3.0%	
P3 window	4.2%		3.8%	4.0%	
A0 window	69.8%	80-90%	83.2%	84.4%	90-96%
A2 window	3.2%	<0.5%	5.3%	3.2	<3.0%

P3: posttranslationally modified hemoglobin.

iron overload in patient III.2 was documented by MRI assessment of the heart and the liver.

Both II.1 and III.2 have developed significant splenomegaly yet without any signs of hypersplenism. The youngest patient (IV.2) does not have a palpable spleen, possibly due to the still very young age.

In the 1960-70s splenectomy was considered an effective therapeutic option for hereditary hemolytic anemias. Over time, it became clear that splenectomy is not effective in all conditions presenting with chronic hemolytic anemia. On the other hand, the resulting asplenia may lead to severe complications. An expert panel has recently issued recommendations regarding splenectomy in hereditary hemolytic anemias.[11] The experts agree that splenectomy should only be considered for patients with unstable Hb who have severe anemia and/or massive or symptomatic splenomegaly. The disease course in patient I.1, who has been splenectomized at the age of 16 years corroborates those recommendations. The effect of the splenectomy seems to have been equivocal, as the original paper reported on improvement,[4] while a subsequent report stated that splenectomy did not significantly influence anemia.[9] Moreover, the latter paper, which reported on ferrokinetic and erythrokinetic nuclide scan findings in the patient and his daughter concluded that spleen does not play any appreciable role in this condition.[9]

Based on the observation of the largest affected pedigree to date, we conclude that Hb Wien is a very rare unstable Hb variant with an autosomal-dominant inheritance pattern, resulting in chronic non-transfusion-dependent microcytic hemolytic anemia. Splenectomy is of equivocal effectiveness in this condition and should be avoided given the increased risk for thrombotic complications.

Sandra Hilbert,[1] Astrid Voill-Glaninger,[2] Beata Höller[2] and Milen Minkov[1,3]

[1]Department of Pediatrics, Neonatology and Adolescent Medicine, Vienna North Hospital – Clinic Floridsdorf; [2]Institute for Laboratory Medicine, Rudolfstiftung Hospital and [3]Sigmund Freud Private University, Vienna, Austria

Correspondence: MILEN MINKOV
milen.minkov@wienkav.at
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