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CASE REPORT

Identification of a Novel Nonsense Mutation in a Patient with Transfusion-Dependent Hb H Disease

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SUMMARY

Background: **Hb H disease is a form of α-thalassemia. The high clinical variability is influenced by the exact combination of mutations. Here we report on a 29-year-old female patient from Afghanistan who received regular blood transfusions since her childhood.**
Methods: **For diagnosis we employed Sanger sequencing, multiplex ligation-dependent probe amplification, hemoglobin-electrophoresis, and hematological analysis.**
Results: **Molecular genetic analysis revealed a non-deletional Hb H genotype with two in cis point mutations in *HBA1* (c.183G>T;p.Lys61Asn and c.184A>T;p.Lys62*) in addition to the common deletions $\alpha^{4.2}$ and $\alpha^{3.7}$ in *HBA2*. The nonsense-mutation p.Lys62* has not been described before. Hematological data were in accordance with the genetic findings.**
Conclusions: **We describe here a novel mutation in the *HBA1* gene and support evidence for non-deletional type of Hb H leading to transfusion-dependent anemia.**
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KEY WORDS

Hb H disease, α-thalassemia, genetics

CASE PRESENTATION

α-thalassemia is among the most frequent autosomal recessive disorders characterized by microcytic hypochromic anemia. The highest prevalence is found in (former) malaria areas like Mediterranean countries, South-East Asia, Africa, the Middle East, and the Indian subcontinent [1]. In these areas a positive selection for thalassemia alleles took place since carriers of thalassemia mutations are protected to some extent against falciparum malaria. Over the last decades, the incidence of α-thalassemia in former non-endemic countries like Germany increased due to demographic changes.
Hemoglobin H (Hb H) disease is a clinically highly variable form of α-thalassemia [1,2]. In case of Hb H disease three of the four *HBA* gene copies are defective. As a consequence, production of the α-globin chains is re-

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Table 1. Hematological and genetic data of the affected patient and two of her sons.

Hematological analysis	Patient (29 years)	Son 1 (8 years)	Son 2 (13 years)
Leucocytes (1000/µL)	12.5	10.4	6.6
Thrombocytes (1000/µL)	597	310	262
Erythrocytes (Mill/µL)	4.9	4.7	5.6
Morphology of erythrocytes	Aniso-, poikilocytosis, Hypo-, polychromasie, target cells, macrocytes	Aniso-, poikilocytosis	Aniso-, poikilocytosis
Hemoglobin (g/dL)	8.6	11.0	11.7
MCV (fL)	74.5	71.3	64.1
MCH (pg)	17.7	23.2	21.0
Hb-electrophoresis	Hb A 95.7% Hb A2 1.0% Hb F n.d. Hb H 3.3%	Hb A 97.5% Hb A2 2.5% Hb F n.d. Hb H n.d.	Hb A 97.6% Hb A2 2.4% Hb F n.d. Hb H n.d.
Genetic analysis			
Alpha-thalassemia genotype	*HBA2*: [deletion -$\alpha^{4.2}$]+ [deletion -$\alpha^{3.7}$] *HBA1*: c.[183G>T; c.184A>T]	*HBA2*: heterozygous deletion -$\alpha^{3.7}$	*HBA2*: heterozygous deletion -$\alpha^{4.2}$ *HBA1*: c.[183G>T; c.184A>T]
Corresponds clinically to	Hb H disease	α-thalassemia minima	α-thalassemia minor

n.d. - not detected.

duced leading to an imbalance in globin chain composition and the formation of unstable β-chains aggregates, called Hb H. Hb H disease has long been thought to be a rather mild disease since most patients can compensate hemolytic anemia with hemoglobin levels of more than 9 g/dL. These patients rarely become transfusion dependent.

So called α-thalassemia carriers, who carry one or two defective *HBA* copies are generally clinically unaffected but have microcytic and hypochromic erythrocytes. Individuals with Hb H disease carry three defective *HBA* copies. The clinical manifestation shows a high variability. The most severe form of this condition is Hb H hydrops fetalis. It has been described that patients carrying a point mutation in addition to deletions, so called non-deletional Hb H, have a more severe clinical phenotype than those patients that carry only deletions [2]. Infants with Hb Bart´s hydrops fetalis syndrome (all four *HBA* copies are defective) almost always die *in utero*. Hb H disease may be critical especially during certain conditions like infections and also pregnancy, since both, pregnancy and thalassemia, are associated with an increased risk of thromboembolism [4]. Even without any clinically overt thromboembolic complication (deep vein thrombosis, pulmonary embolism) in the history of our patient, the thromboembolic risk is additionally increased due to protein Z deficiency and an elevated lipoprotein-a level (data not shown).

We report on a 29-year-old female patient from Afghanistan (Herat) who received regular blood transfusions (every three months) since her childhood. She had a splenectomy in May 2013 at the age of 25 years. The patient is in good health with a body height of 158 cm and a weight of 72 kg.

She was pregnant four times and gave birth to three boys. Data of two sons are shown in Table 1. In September 2016 she gave birth to a third boy who has not yet been genotyped. Moreover, she had an early abortion in the 6th week of pregnancy in 2013. Her husband is the cousin of her father and was not diagnosed with thalassemia.

RESULTS

Hematological analysis showed that the patient had microcytic and hypochromic anemia and signs of a mild iron overload (ferritin serum level of 390 ng/mL in absence of an acute phase reaction; reference range: 13 - 150 ng/mL).

Using high-resolution Hb-electrophoresis (Sebia Capillary Electrophoresis) 3.3% abnormal hemoglobin was observed in zone 15, which corresponds to the presence of Hb H.

In order to characterize α-thalassemia in more detail we performed molecular genetic analysis by sequence analysis of the genes *HBA1* and *HBA2* (exons and introns). For the identification of large deletions in the α-globin

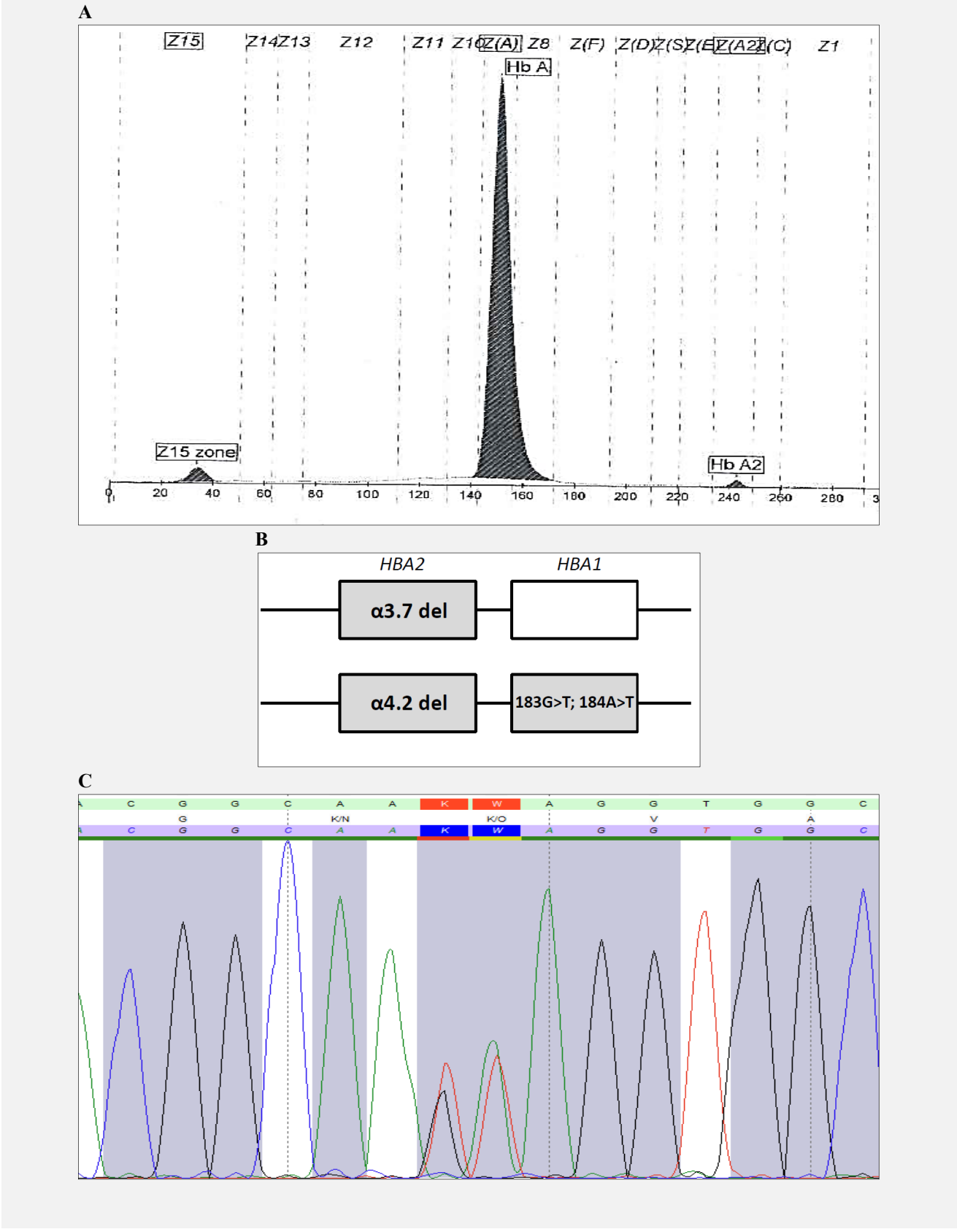

Figure 1. A. Hb electrophoresis showing the abnormal Hb H peak of 3.3% in zone Z15. B. Schematic depiction of allelic distribution of the Hb H patient mutations. Inactivated gene copies are shown in grey. C. Electropherogramm of DNA sequencing analysis showing the two adjacent point mutations in one copy of the *HBA1* gene.

locus we employed multiplex ligation-dependent probe amplification (MRC-Holland). The patient lacked the *HBA2* gene due to the combination of two common deletions, namely $-\alpha^{4.2}$ and $-\alpha^{3.7}$. Moreover, sequencing identified the variants c.183G>T;p.Lys61Asn and c.184A>T;p.Lys62*, which localize to exon 2 of the *HBA1* gene. The first variant has been described before and is known as Hb Zambia (HbVar ID 88). The second variant is a nonsense-mutation and has not been described before. In any case, this patient will inherit one mutant allele to all of her children since both maternal alleles carry the mutations. Both sons are thus α-thalassemia carriers (Table 1).

DISCUSSION

Hemoglobinopathies are among the most frequent monogenic diseases worldwide. Although hemoglobinopathies are rarely seen in individuals that originate from Germany, there is an increasing number of thalassemia and other hemoglobinopathies in Germany due to migration of individuals from countries with a high frequency of these alleles [3].

We report on a 29-year-old woman with transfusion dependent Hb H disease as diagnosed by clinical symptoms, hematologic analysis, Hb-electrophoresis, and genetic analysis. It should be noted, that abnormal Hb H peaks can be easily overseen. Due to the unstable nature of Hb H the respective peaks can be very small. Thus, genetic analysis should be performed whenever there is a suspicion of Hb H disease.

We identified three known mutations and the novel nonsense-mutation c.184A>T;p.Lys62*, which localizes to exon 2 of the *HBA1* gene. Most likely the RNA produced from this allele is subject to nonsense mediated decay and thus no production of α-globin chain can be expected from the affected *HBA1* allele. The two adjacent point mutations c.183G>T;p.Lys61Asn and c.184A>T;p.Lys62* must be located on the same allele, since both mutations were inherited together to the 13-year-old son together with the heterozygous deletion $-\alpha^{4.2}$ of the *HBA2* gene.

CONCLUSION

Hb H disease is highly variable in its clinical presentation. Among other factors specific combinations of mutations are thought to modulate the severity of the disease. Here we describe a novel point mutation in the *HBA1* gene which is likely to contribute to an - even occasionally - transfusion dependent form of Hb H disease. Our observation supports the accumulating evidence for a stronger clinical phenotype in patients with non-deletional mutations.

Declaration of Interest:
The authors disclose there are no commercial or other associations that might pose a conflict of interest in connection with the submitted article.

References:

1. Harteveld CL, Higgs DR. Alpha-thalassemia. Orphanet J Rare Dis 2010;5:13 (PMID: 20507641).
2. Fucharoen S, Viprakasit V. Hb H disease: clinical course and disease modifiers. Hematology Am Soc Hematol Educ Program 2009:26-34 (PMID: 20008179).
3. Kohne E, Kleihauer E. Hemoglobinopathies: a longitudinal study over four decades. Dtsch Arztebl Int 2010;107(5):65-71 (PMID: 20186311).
4. Tongsong T, Srisupudit K, Luewan S. Outcomes of pregnancies affected by hemoglobin H disease. Int J Gynecol Obst 2009;104 (3):206-9 (PMID: 19027901).