Codon 104(-G), a dominant β⁰-thalassemia-like phenotype in a German Caucasian family is associated with mild chronic hemolytic anemia but influenced in severity by co-inherited genetic factors

Codon 104(-G), a heterozygous frameshift mutation in exon 2 of HBB, resulted in a dominantly inherited β⁰-phenotype with mild anemia in a German kindred, and thalassemia intermedia in the index patient. A co-inherited α gene triplication, long-term transfusion therapy, and ineffective erythropoiesis were confounding factors.

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We report a β⁰-thalassemia deletion mutation with dominant mode of inheritance in a German Caucasian family of Huguenot descent (Figure 1). The proposita (IV-5) presented in our hospital (2002) with hepatosplenomegaly and a Thalassemia intermedia phenotype. Erythrocytes had been previously transfused to her (300 units) and her brother (IV-2, 500 units). Her brother died of malignant melanoma in 2003. Transfusion therapy was continued in the proposita up to the time, when iron overload became evident. From 1999, she had been treated with erythropoietin and iron chelation therapy. Her sister (IV-7) and daughter (V-4) presented with mild chronic hemolytic anemia. Elevated HbA_2 (3.9-5.1%) and HbF-levels (3.8 and 5.1%) in the proposita and her daughter (V-4) were detected by high performance liquid chromatography on an ÄKTA™ basic 10A system (Amersham Biosciences, Freiburg). Low values for Hb (7.7-11.2 g/dL), Hct (28-35%), MCV (69-73.2 fl) and MCH (22.1-24.2 pg) indicated hypochromic anemia (Table 1). Erythrocyte enzyme activities for glycolysis and pentose phosphate cycle were normal. Elevated serum concentrations of iron (39.3 µmol/L), ferritin (2016 µg/L) and a transferrin saturation of 89.8% denoted iron overload and/or hemochromatosis in the proposita. Ultrasonography and computerized tomography showed increased hepatic density, a clinical sign of iron overload. The absence of aberrant or unstable hemoglobin fraction in biochemical Hb analyses together with a thalassemia-like clinical picture suggested β⁰-thalassemia. Informed consent was given for genetic analyses. DNA extraction and polymerase chain reaction (PCR) analysis were performed using standard methods, sequencing with a CEQ Quick Start Kit on a CEQ 8800 automated DNA Sequencer (Beckman Coulter, Krefeld). Primers and PCR conditions are available from the authors upon request. The family members were heterozygous for a β-thalassemia mutation [codon 104(-G)] at the exon2/IVS-II boundary within a homonucleotide run of three guanines (494-496; -G) (Figure 2A). The deletion mutant at the end of

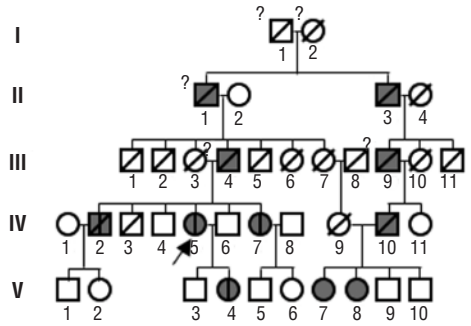

Figure 1. Pedigree of German kindred shows segregation of dominantly transmitted β-Thalassemia. The proposita is marked by an arrow; ⊘: dead; ●: dominant Thalassemia; ○: healthy; ◐: heterozygous; ?: unconfirmed disease.

Table 1. Hematological data of four members of German index family (after discontinued transfusion therapy of members IV-2 and IV-5).

	Pedigree No.				
	IV-2	IV-5 (proposita)	IV-7	V-4	normal values
sex -born [year]	M-1925	F-1933	F-1938	F-1963	
HbA (%)	94.9	92.2	96	91	97.5
HbA2 (%)	5.1	4.0	4.0	3.9	2.5
HbF (%)	–	3.8	–	5.1	< 2
Hb (g/dl)	11.2	7.7	9.1	10.6	12-18
Hct (%)	35	24	28	32	36-49
MCH (pg)	22.1	22.7	23.5	24.2	27-33
MCV (fl)	69	73.2	73	72	80-96
MCHC (g/dl)	32.1	32.3	32	33.5	32-36
RDW-CV (%)	26.9	31.4	17.4	28.4	15.8 +/- 2.9
RBC ($x10^{12}$/l)	ND	3.91	3.96	4.51	4.2-6.3
Reti (%)	2.4	2.1	3.6	1.5	0.5-2
transfusions given	Yes	Yes	No	No	
serum iron (µmol/l)	ND	39.3	11.1	16.6	6.3-30.1
ferritin (µg/l)	ND	2016	148	78	30-300
transferrin saturation (%)	ND	89.8	20.7	35.5	13.1-44.5
total bilirubin (mg/dl)	ND	3	1.3	1.3	0.3-1.9
direct bilirubin (mg/dl)	ND	0.9	0.5	0.5	0- 0.3
haptoglobin (mg/dl)	ND	6	22	?8	27-139
LDH (U/l)	ND	327	ND	275	120-240
HFE-H63D	ND	het (H/D)	het (H/D)	het (H/D)	wt: H
HFE-S65C	ND	wt	wt	wt	wt: S
HFE-C282Y	ND	wt	wt	het (C/Y)	wt: C
α globin gene number	ND	αα/ααα	αα/αα	αα/αα	αα/αα
$A(TA)_nTAA$ motif in UGT1A1 promoter	ND	6/7	6/7	5/6	5/6 or 6/6
-158 (C/T) G-γ promoter	ND	C/T	C/T	C/T	C

M: male; F: female; Hct: hematocrit; MCH: mean corpuscular haemoglobin; Reti: reticulocytes; RDW-CV: coefficient of variation of red cell distribution width; ND: not determined; LDH: lactate dehydrogenase; HFE gene mutations: H63D, S65C and C282Y; het: heterozygous, wt: wild type, UGT1A1: UDP-glucuronosyltransferase 1A1 gene.

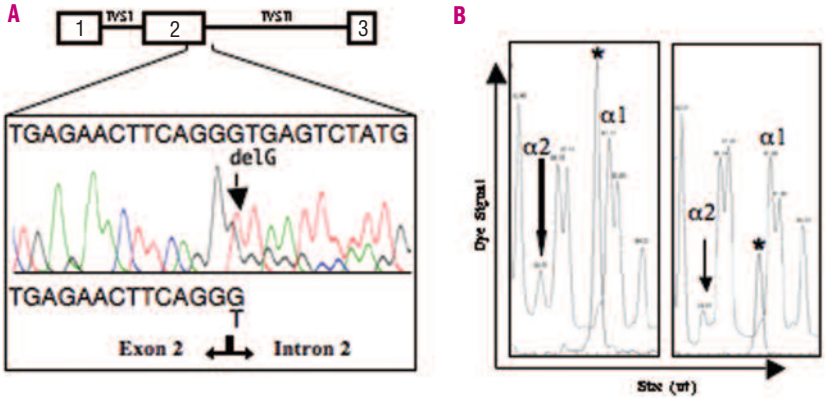

Figure 2. β- and α globin gene analyses. **A.** Sequence analysis of the PCR-amplified exon2/3'end-region of β globin. Above the electropherogram a schematic drawing of exon/intron composition is shown. Genomic DNA from proposita shows a heterozygous G deletion at the end of exon 2 (exon2/IVS-II splice site). **B.** Peak profiles of *HBA* gene cluster MLPA-analysis. Left panel: a triplicated α2-peak (ααα) is present in the proposita (IV-5). Right panel: a wild type α2-peak (αα) is present in her daughter (V-4); [*] internal size marker; α2-peaks are indicated by an arrow.

exon 2 was similar to the dominant β0-deletion mutant Hb Manhattan [codon 109(-G)] at the beginning of exon 3.[1] Both mutation-dependent modified C-terminal sequences extend to 156 amino acids and include an identical 48-residue-long terminal tail sequence from codon 109 up to codon 156. The –1 frameshift led to the introduction of hydrophobic residues and five additional prolines, which compromise the formation of a normal H-helix. The unstable polypeptide was not detectable, probably due to rapid metabolization.[1] Mutant β globin mRNA, and erythrocyte inclusion bodies were also undetectable. Coexistence of a triplicated α gene enhances globin chain imbalance and may be associated with more severe β-thalassemia features.[2,3] α globin gene amplification in the proposita was detected by MLPA-analysis (seen as an increase in the α2-peak), using the MLPA α-globin kit (ServiceXS; *http://www.servicexs.com*) with adapted fluorescent oligonucleotides and the CEQ 8800 DNA Sequencer (Figure 2B). Deposition of excessive α chains in erythroid precursors in the bone marrow may have led to accelerated apoptosis and ineffective erythropoiesis.[4,5] Anemia and ongoing hemolysis (elevated serum concentrations of unconjugated bilirubin and lactate dehydrogenase and a suppressed level of haptoglobin) in the presence of only a moderate reticulocytosis (2.1%) suggest an impeded erythropoiesis. A bilirubin UDP-glucuronosyltransferase 1A1 (UGT1A1) promoter polymorphism may be associated both with an unconjugated bilirubin level and an elevated risk of cholelithiasis. The TA repeats (5, 6, 7, or 8) in the TATA box are inversely correlated with gene transcription efficiency and overall enzyme activity.[6] Indeed, the proposita and her sister (IV-7), who were both cholecystectomized, displayed a $(TA)_6/(TA)_7$ genotype. The $(TA)_5/(TA)_6$ genotype of the daughter (V-4) was associated with normal bilirubin concentrations and a stone-free gallbladder.[7] The polymorphic single-base substitution -158 $^{C}\gamma$(C→T; XmnI polymorphism) appears to cause an increase of HbF especially during erythropoietic stress, partially compensating for absent β-chain synthesis with consequent amelioration of globin chain imbalance and a milder phenotype.[8,9] However, since, despite heterozygosity in three family members, elevated HbF-values (3.8 and 5.1%) were only detected in the proposita and her daughter this remains open to debate (V-4; Table 1). Co-inheritance of a heterozygous H63D mutation in the hereditary hemochromatosis (HFE) gene may contribute to severe iron overload in β-thalassemia minor.[10] However, the presence of a heterozygous H63D mutation in three family members, but iron overload only in the proposita who had received multiple blood transfusions, indicates that it was mainly blood transfusion therapy along with ineffective erythropoiesis that caused secondary hemochromatosis.

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