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Red Cell Disorders

A new polyadenylation site mutation associated with a mild β-thalassemia phenotype

At least 180,000 autochthonous and allochthonous people are carriers of a large spectrum of hemoglobinopathies in The Netherlands.[1] We describe two cases, the first, a 33-year old Surinamese Creole woman, studied because of an intermediate hemolytic anemia; the second, a couple requesting analysis because of a previously diagnosed carrier state in the male partner, while the carrier state in the pregnant female was uncertain.

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Hematologic parameters were measured on fresh EDTA blood on a semiautomatic counter and lysates were examined on alkaline starch gel electrophoresis. The HbA2 and HbF levels were estimated by automatic high performance liquid chromatographic (HPLC) analysis (Variant I and Variant II, Bio-Rad Laboratories, Hercules, CA, USA). Biochemical and molecular analyses were done according to previously described procedures.[2-4] Samples were analyzed on an ABI Prism™ 377 sequencing apparatus (Applied Biosystems, Perkin Elmer Corporation, Foster City, Ca, USA). The haplotype of the β-genes was determined using the following 7 polymorphic sites and restriction enzymes: 1; 5'-ε (Hinc II), 2; Gγ (Hind III), 3; Aγ (Hind III), 4; Ψβ (Hinc II), 5; 3'- Ψβ (Hinc II), 6; 5'-β, (Hinf I), 7; 3'-β (Hinf I) according to Varawalla *et al.*[5] and Sutton *et al.*[6]

Family #1. A 33-year old Surinamese woman (II-3), her mother (I-2) and two older sisters were examined because of a chronic hypochromic microcytic hemolytic anemia. The proband (II-3) presented with the parameters of an intermediate β-thalassemia. Her mother was microcytic but not anemic with a strongly elevated HbF level (58%).

Table 1. Hematologic, biochemical and molecular data in family 1.

Parameters	*I-1*	*I-2*	*II-1*	*II-2*	*II-3**
Age/gender	?/M	69/F	50/F	48/F	33/F
Hb (g/dL)	n.d.	13.5	13.5	12.6	10.8
PCV (l/L)	n.d.	0.40	0.41	0.40	0.34
RBC (10^{12}/L)	n.d.	6.25	5.8	4.9	5.7
MCV (fL)	n.d.	64	71	80	60
MCH (pg)	n.d.	21.6	22.9	25.3	19.0
Hb A_2 (%)	n.d.	4.6	5.7	2.1	8.56
Hb F (%)	n.d.	58	1.2	12	3.6
Osmotic fragility	n.d.	↓↓↓	↓↓↓	↓↓↓	↓↓↓
Haptoglobin (mg/dL)	n.d.	30	265	76	44
Ferritin (μg/L)	n.d.	Normal	Normal	Normal	Normal
Inclusion bodies	n.d.	n.d.	n.d.	n.d.	n.d.
Erythr. morphology	n.d.	++	+	+	+++
ZPP (μmoL/mol heme)	n.d.	n.d.	n.d.	n.d.	n.d.
β/α synth. ratio	n.d.	n.d.	0.7	0.6	0.22
Gγ (%)	n.d.	54	n.d.	54	n.d.
Aγ (%)	n.d.	46	n.d.	46	n.d.
β defect 1	→AATATA	δβdel	-88 (C→T)	δβdel	→AATATA
β defect 2		-88 (C→T)			-88 (C→T)
β-cluster haplotype					
Fragment					
1	–/–#	+/–	+/–	–/–	+/–
2	–/–	–/–	–/–	–/–	–/–
3	–/–	–/–	–/–	–/–	–/–
4	–/–	–/–	–/–	–/–	–/–
5	+/+	+/+	+/+	+/+	+/+
6	+/+	+/+	+/+	+/+	+/+
7	+/–	+/+	+/+	+/+	+/–

**proband; #: deduced; n.d.: not done; +,++,+++: degree of abnormality.*

The proband's two older sisters had the phenotypes of heterozygous β-thalassemia (II-1) and of a mild δβ-thalassemia trait with increased HbF level (II-2). The father (I-1) was not available for examination. Direct sequencing revealed the –88 (C→T) β^+ thalassemia mutation[7] and the new poly A mutation AATAAA→AATATA. These data are summarized in Table 1.

Family #2. A Surinamese couple was referred for risk assessment after the male partner had been diagnosed as a thalassemia carrier. The carrier state of the male was easily established and confirmed at the molecular level as a –29 (A→G) mutation in the presence of α^+ thalassemia heterozygosity. His pregnant partner presented non-thalassemic indices and a normal or a slightly elevated HbA_2 level depending on the HPLC system used for measurements. After sequencing the woman was found to be a carrier of the same poly A mutation previously found in family #1. The DNA of the fetus showed no thalassemia mutations. Haplotype analysis revealed that the poly A mutation was associated with the same haplotype (– – – – + + –) in both families (Tables 1 and 2). These data are summarized in Table 2.

The proband (II-3) in family #1 had an intermediate phenotype caused by the β^+ -88 C→T and the new polyadenylation site mutation, which destabilizes an otherwise normal mRNA product. The proband's mother (I-2) showed a false homozygous pattern for the -88 mutation, and a very high HbF expression (58%), without anemia or hemolysis. A coexisting $G\gamma A\gamma(\delta\beta)^0$ deletion, called HPFH-2, was confirmed (Table 1).

The routine analysis of the couple (family #2) confirmed a typical β thalassemia minor phenotype in the man and a

Table 2. Hematologic, biochemical and molecular data in family #2.

Parameters	*I-1*	*I-2*	*II-1*
Age/gender	24/M	24/F	-20 week/?
Hb (g/dL)	14.8	11.9	n.d.
PCV (l/L)	0.48	0.33	n.d.
RBC (10^{12}/L)	6.05	4.8	n.d.
MCV (fl)	80	68	n.d.
MCH (pg)	24.5	24.8	n.d.
Hb A_2 (%)	5.6-4.1	4.3-3.0	n.d.
Hb F (%)	1.9	0.5	n.d.
Osmotic fragility	↓↓↓	↓	n.d.
Haptoglobin (mg/dL)	93	63	n.d.
Ferritin (μg/L)	n.d.	n.d.	n.d.
Inclusion bodies	Absent	Absent	n.d.
Erythrocyte morphology	+	++	n.d.
ZPP (μmol/mol heme)	24	33	n.d.
β/α synth. ratio	n.d.	n.d.	n.d.
Gγ (%)	n.d.	n.d.	n.d.
Aγ (%)	n.d.	n.d.	n.d.
β defect	-29 (A→G)	→AATATA	Absent
α defect	$-\alpha^{3.7}/\alpha\alpha$	Absent	Absent
β-Cluster haplotype			
Fragment			
1	–/–	+/–	–/+
2	+/+	+/–	+/+
3	–/–	–/–	–/–
4	+/+	–/–	+/–
5	+/+	+/+	+/+
6	+/+	+/+	+/+
7	+/+	+/–	+/+

n.d. = not done; +,++,+++ = degree of abnormality.

doubtful phenotype in the woman. This case shows that combined hematologic, biochemical and molecular analyses are needed for risk assessment for β-thalassemia. The HbA_2 levels were elevated (5.6%) and slightly elevated (4.3%) in the man and in the woman, respectively, using the Variant I HPLC, although when the Variant II was used the values were elevated (4.1%) and border line (3.0%), respectively. A retention time of 3.65 minutes was associated with elevated values in both patients using the Variant I. A shorter retention time of 3.50 minutes gave lower values for both patients using Variant II. The optimal retention time of 3.65 minutes on Variant II could be reached by lowering the temperature to 28°C; at this temperature the HbA_2 levels of both patients were measured comparably on both apparatuses. In conclusion, a new poly A mutation (AATAAA → AATATA) was found in two unrelated families on the same rare (– – – – + + –) haplotype, indicating a common African origin of the two mutations that can be classified as a β^+ defect. A borderline HbA_2 value does not exclude a β-thalassemia defect.

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Red Cell Disorders

Nutritional iron status in children with α^+thalassemia and the sickle cell trait in a malaria endemic area on the coast of Kenya

Although hemoglobinopathies such as α^+thalassemia and the sickle cell trait might contribute to anemia in African children, we hypothesized that they might also enhance iron absorption under circumstances of critical availability, and that this could attenuate their hematologic effects. We found no support for this hypothesis in a cohort of children living on the coast of Kenya.

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Iron deficiency, malaria and the hemoglobinopathies, particularly α^+thalassemia, are important causes of anemia in many tropical populations, but their interactions are poorly understood. For example, α^+thalassemia protects against severe and complicated *P. falciparum* malaria,[1] but does not protect against the milder forms,[2] which are probably most relevant to steady-state hemoglobin concentration at a population level. Moreover, some forms of thalassemia lead to iron loading[3,4] due to increased iron absorption,[4] an observation that has led us to speculate that some of the asymptomatic forms of α^+thalassemia might protect against iron deficiency.[5] We therefore investigated this question on the coast of Kenya.

The study included 270 children who were involved in a cohort study investigating the immunology of malaria.[6] Children were sampled during a cross-sectional survey conducted in May 2002. We took a ferritin-based approach to the classification of iron status, on the grounds that, unlike soluble transferrin receptor,[5] ferritin metabolism is not affected by clinically silent hemoglobinopathies, such as HbAS and α^+thalassemia. The main concern regarding the use of ferritin is its potential lack of