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

Table 1. Biological and biochemical characteristics of study children by α⁺thalassemia genotype and hemoglobin type.

	α⁺thalassemia genotype					Hemoglobin type		
	Normal (αα/αα)	Heterozygote (-α/αα)	p	Homozygote (-α/-α)	p	HbAA	HbAS	p
N (%)	80 (30.2)	137 (51.7)		48 (18.1)		229 (85.4)	39 (14.6)	
Age (months)[a]	47.7 (22.9)	52.4 (23.7)	0.15	44.1 (23.0)	0.40	50.2 (23.9)	45.8 (22.4)	0.28
F/M ratio	0.5	0.9	0.13<	1.0	0.07	0.71	1.31	0.05
P. falciparum[b,c]	18/75 (24.0)	26/134 (19.4)	0.61	3/45 (6.7)	0.05	40/219 (18.3)	8/38 (21.1)	0.91
CRP >10 mg/L[c]	11/80 (13.8)	10/135 (7.2)	0.15	2/48 (4.2)	0.13	19/229 (8.3)	4/39 (10.3)	0.76
Iron (μmol/L)[a]	7.79 (4.63)	7.67 (4.96)	0.66	6.68 (4.17)	0.12	7.77 (4.89)	6.32 (3.37)	0.08
Ferritin (μg/mL)[d]	20.42 (16.67-25.12)	15.85 (13.68-18.97)	0.25*	17.38 (12.88-23.99)	0.20*	18.20 (15.85-20.42)	13.58 (10.47-17.78)	0.04*
Transferrin (g/L)[a]	2.82(0.48)	2.88 (0.60)	0.40	2.84 (0.60)	0.79	2.86 (0.58)	2.82 (0.48)	0.67
% transferrin saturation[a]	11.99 (7.19)	11.53 (8.08)	0.69	9.81 (6.60)	0.10	11.64 (7.78)	9.42 (5.90)	0.10
Iron deficient[e,f]	23/72 (31.9)	52/132 (39.4)	0.36	17/46 (40.0)	0.55	77/216 (35.7)	16/37 (43.2)	0.46
Iron replete[g]	31/72 (43.0)	55/132 (41.7)	0.88	26/46 (56.5)	0.19	99/216 (45.8)	15/37 (40.5)	0.60
Iron status unclassified[h,e]	18/72 (25.0)	25/132 (18.94)	0.37	3/46 (6.5)	0.01	40/216 (18.5)	6/37 (16.2)	0.82

*[a]Mean (SD); [b]Data are missing for some children; [c]proportion of group (%); [d]geometric mean (95% CI). [e]Only children with full data on ferritin, CRP, blood smear and fever are included in the classification of iron status. [f]ferritin <12 μg/mL, [g]ferritin >12 μg/mL, CRP< 10 ng/L, no fever and blood smear negative for P. falciparum malaria; [h]ferritin >12 μg/mL but either febrile or CRP>10 mg/L or blood smear positive for P. falciparum malaria. Means compared between each genotype separately with normals by unpaired Student's t tests with the exception of * where significance values were derived by multiple linear regression, adjusting for hemoglobin type (HbAA, HbAS), α⁺thalassemia genotype (normal, heterozygous, homozygous), age (in 2-year blocks), sex, fever (> or ≤ 37.5°C), malaria parasite positivity, and CRP (<10, ≥10mg/L). Proportion data were compared by Fisher's exact test. Thalassemia typing was unsuccessful in 5 children and HbAS typing was omitted in 2. CRP: C-reactive protein.*

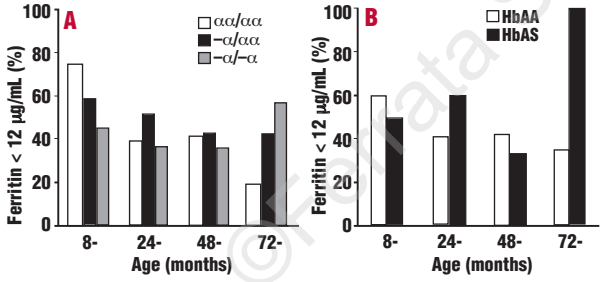

Figure 1. Iron deficiency was defined as a plasma ferritin concentration <12 μg/mL. Children with a plasma ferritin ≥12 μg/mL were excluded from the denominator if they were febrile, had a positive malaria blood film or had a plasma CRP concentration of ≥10 mg/L). Total number of children in each age and genotype category from left to right: A 12, 22, 13, 15, 27, 11, 12, 33, 14, 15, 27, 7; B 40, 6, 46, 10, 47, 12, 46, 4.

sensitivity in identifying iron deficiency: children who are actually iron deficient may be categorized as iron replete if their plasma ferritin is falsely raised due to concomitant malaria or inflammation. We took account of this by including inflammatory markers and malaria in our regression-based analyses, and by excluding children from our categorical analysis whose ferritin was ≥12 μg/mL but who were febrile (an axillary temperature <37.5° C), had evidence of inflammation (a C-reactive protein ≥10mg/mL; the upper limit of the reference range for the assay), or had a blood film positive for malaria parasites. HbAS was typed by electrophoresis, and α⁺thalassemia by polymerase chain reaction.[7] The only type of α⁺thalassemia that occurs commonly in the study area is the $-\alpha^{3.7}$kb deletion; we detected no other genotypes in a representative group of 200 children sampled from around the entire KEMRI study area (*Williams, unpublished observations*).

We found no significant between-genotype differences in any biological characteristics on univariate analysis (Table 1) with three exceptions: the parasite prevalence was significantly lower in homozygotes for α⁺thalassemia than in normal children, the proportion of females was significantly higher in the HbAS than the HbAA group, and iron status was unclassifiable in fewer α⁺thalassemia homozygous than in normal children. We investigated the effect of α⁺thalassemia and HbAS on ferritin concentration by multivariable linear regression, fitting log ferritin as the dependent variable and hemoglobin type, α⁺thalassemia genotype, age (in 2-year blocks), sex, fever (> or ≤37.5°C), malaria parasite positivity (0,1), and C-reactive protein, as explanatory variables. Neither −α/αα nor −α/−α was significantly associated with log ferritin concentration (β=-0.08; −0.22,0.05; p=0.245 and β=-0.11; −0.29,0.06; p=0.201 respectively), and HbAS was negatively associated (β=−0.20; −0.38,−0.02; p=0.037). Finally, we investigated the effect of each hemoglobinopathy on iron deficiency (defined as above) by logistic regression including in our model the explana-

tory variables hemoglobin type, α+thalassemia genotype, age (in 2 year blocks), sex, fever (> or ≤37.5°C), malaria parasite positivity, C-reactive protein, and status unclassifiable (0,1). We found no evidence for an association between either hemoglobinopathy and protection against iron deficiency: the adjusted ORs for –α/αα and for –α/–α were 1.51 (0.76,3.01; p=0.239) and 0.94 (0.40,2.20; p=0.880), respectively and the OR for HbAS was 1.27 (0.55,2.90; p=0.572). Moreover, while the prevalence of iron deficiency declined with age in completely normal children (αα/αα with HbAA) [OR 0.28 in children <24 months compared to children <24 months of age (0.08,0.94); p=0.039; n=54], it did not decline in either heterozygous [OR 0.47 (0.18,1.23); p=0.124; n=94], or homozygous [OR 1.03 (0.23,4.53); p=0.967; n=37] α+thalassemia. Too few children had the genotype αα/αα and HbAS (n=6) to allow for meaningful interpretation. Although data on biochemical iron markers have been presented for both α+thalassemia and HbAS in several African populations,[8] it is difficult to interpret such data in the absence of information on fever, inflammatory markers, or malaria parasitemia. In the current study we took account of these parameters, and found no evidence that either condition protects against iron deficiency. While it is well established that both HbAS and α+thalassemia are strongly protective against severe *P. falciparum* malaria,[1,9] susceptibility is also affected by a range of other host-related factors. There is some evidence to suggest that iron deficiency may protect against malaria,[10] a hypothesis supported by our recent observations on the coast of Kenya.[6] In this context, the trend we found towards reduced iron status in both conditions is interesting - if this holds up in further studies it could constitute one mechanism by which these conditions result in malaria protection.

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Key words: thalassemia, HbAS, iron status, malaria.

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Immunodeficiency Syndromes

Neutropenia in Iranian patients with primary immunodeficiency disorders

Neutropenia may occur in some of the primary immunodeficiency disorders. We reviewed the records of 56 neutropenic patients. The most common disorders were Shwachman-Diamond syndrome, cyclic neutropenia, Kostmann disease, Chediak-Higashi syndrome, hyper IgM syndromes, severe combined immunodeficiency, hyper IgE syndrome, and common variable immunodeficiency.

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Primary immunodeficiency disorders (PiD) are relatively rare disorders, characterized by an unusual susceptibility to infections.[1,2] Some conditions with PiD may also feature neutropenia as a consequence of either an intercurrent infection or an autoimmune disease.[3,4] The present study reports the clinical and laboratory findings of Iranian PiD patients with associated neutropenia from a main immunodeficiency referral center in Iran.

Four hundred and seventy-four patients with the diagnosis of PiD have so far been referred to the Iranian Primary Immunodeficiency Registry (IPIDR) during a 24-year period (1980-2004).[2] A review of the clinical records of all these patients identified 56 (11.8%) with associated neutropenia. These patients' data were collected by interview and review of their medical documents.

The characteristics of the 56 cases (32 males, 24 females) with associated neutropenia are shown in Table 1. The mean age of these patients with neutropenia was 10.7±5.7 years (range: 2-25 years), and they were followed through a period of 7.9±4.6 years. The median age at the onset of the PiD disorder was 6.5 months (1-134). The median age at the time of PiD diagnosis was 3 years (2 months-13 years), with a median diagnosis delay of 23 months (<1 month - 12 years). Thirty-nine out of these patients are alive, 10 patients could not be located since one year previously, and the remaining 7 patients have