PCR product has a distinct Tm, depending on base substitutions, single point mutations and insertions-deletions.[3] The PCR was performed on the 5700 Sequence Detection System (PE Biosystems). The analyzed samples were run in quadruplicate and in 3 different PCR runs. No template controls (NTCs) were run in order to verify non-specific PCR product formation.

For genotyping the mutations C282Y and H63D, genomic DNA was run in a primer optimization matrix (primers' concentrations ranging from 50 to 300mM). Forward/reverse primer combinations 50/300, 50/50 and 300/50 gave no aspecific products. Using these primer combinations in the presence of target DNA, the PCR product was detectable after 27, 34 and 30 cycles, respectively. Because lower cycle thresholds are related to a higher sensitivity of the test, the 50/300 mM combination was chosen. Three different PCR reactions were done for each sample, and each amplification was repeated four times. The resulting Tm profiles of three representative genotypes at codon 63 are shown in Figure 1.

The mutations found in 12 individuals are summarized in Table 1. For each subject, the HFE genotype and the Tm mean values (± standard deviation) are shown. We successfully characterized the mutant alleles of the patients, confirming the data obtained by PCR-RLFP (data not shown). The homozygous C282Y genotype shows a Tm mean value of 84.7°C, distinct from those of heterozygous (83.8°C) and wild type homozygous (83.0 °C) individuals. Also for position 63, different Tm mean values characterize mutant homozygous (86.2°C), heterozygous (85.4°C) and wild type homozygous (88.0°C) subjects.

We have developed an easy, inexpensive, automated method for determining the genotypes of the two mutations typical of hemochromatosis. The method is not dependent on fluorescent probes and eliminates post-PCR processing. The reproducibility of the method allowed us to test several subjects; all the samples were successfully and clearly genotyped; the Tm genotyping study was confirmed by PCR- RLFP.

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Table 1. Tm profiles in 12 individuals with different HFE genotype at position 282 and 63.

Cases	Genotype of codon 282	Genotype of codon 63	Tm at position 282 mean value (°C)	Tm at position 282 SD	Tm at position 63 mean value (°C)	Tm at position 63 SD
1	+/+	-/-	84.70	0.03	88.00	0.07
2	+/+	-/-	84.70	0.03	88.00	0.01
3	-/-	+/+	83.00	0.00	86.20	0.02
4	-/-	+/+	83.00	0.10	86.20	0.01
5	+/-	+/-	83.80	0.18	85.40	0.10
6	+/-	+/-	83.80	0.02	85.40	0.02
7	+/-	-/-	83.80	0.15	88.00	0.10
8	+/-	-/-	83.80	0.20	88.00	0.20
9	-/-	+/-	83.00	0.01	85.40	0.12
10	-/-	+/-	83.00	0.02	85.40	0.10
11	-/-	-/-	83.00	0.56	88.00	0.02
12	-/-	-/-	83.00	0.11	88.00	0.20

Tm: Melting tempetature; SD: Standard deviation; +: mutant allele; -: wild type allele.

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References

1. Bacon BR, Olynyk JK, Brunt EM, Britton RS, Wolff RK. HFE genotype in patients with hemochromatosis and other liver diseases. Ann Intern Med 1999; 130:953-62.
2. Ririe KM, Rasmussen RP, Wittwer CT. Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. Anal Biochem 1997; 245: 154-60.
3. Germer S, Higuchi R. Single-tube genotyping without oligonucleotide probes. Genome Res 1999; 9:72-8.

Screening for (--^{SEA}) α-globin gene deletion in β-thalassemia carriers and prevention of hydrops fetalis

In Southeast Asia, where thalassemia is prevalent, heterozygous carriers of both β-thalassemia mutation and the (--^{SEA}) α-globin gene deletion are encountered. These individuals are potentially at risk of being parents to off-springs affected by β-thalassemia major, hemoglobin (Hb) H disease, and Hb Bart's hydrops fetalis. We propose screening for the (--^{SEA}) α-thalassemia mutation in all β-thalassemia carriers to prevent hydrops fetalis.

Sir,

Hb Bart's hydrops fetalis, caused by deletion of all four α-globin genes, is associated with a dismal prognosis for the affected fetus, who will usually die in utero during the third trimester or shortly after birth.[1] Moreover, there is an increased incidence of serious maternal complications in these pregnancies. Preg-

Table 1. Findings in individuals confirmed by DNA-based genotyping to be heterozygous carriers of both β-thalassemia mutation and (—^{SEA}) α-globin gene deletion.

Case	Sex/Age	Hb (g/dL)	MCV (fL)	MCH (pg)	HbA_2 (%)	HbF (%)	HbH bodies	β-globin gene mutation	Family history	Status of partner
1.	F/27	10.2	72.1	23.7	5.5	1.2	negative	codon 17(A→T)	negative	normal
2.	F/30	11.6	70.1	22.6	5.0	1.1	negative	IVSII-654(C→T)	negative	α^0-thalassaemia carrier
3.	F/38	12.6	68.1	21.7	5.4	0.6	positive	IVSII-654(C→T)	negative	unknown
4.	F/29	12.1	70.6	23.0	5.2	1.4	negative	IVSI-1(G T)	positive	α^0-thalassaemia carrier
5.	M/32	17.2	76.6	24.2	4.9	0.4	negative	codons 41/42(-CTTT)	negative	normal
6.	F/34	11.4	71.3	21.5	4.9	1.4	negative	codon 17(A→T)	positive	normal
7.	F/22	12.6	75.7	23.6	5.8	0.6	negative	codons 41/42(-CTTT)	negative	normal
8.	M/4	12.4	65.1	21.0	5.8	0.5	negative	IVSII-654(C→T)	positive	not applicable

Key: Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin

nancies with fetuses affected by Hb Bart's hydrops fetalis syndrome are most commonly seen in couples of southeast Asian ancestry as a result of a high carrier rate of (--^{SEA}) α-globin gene deletion, varying between 4 to 14% in different populations in the region.[1] In order to prevent Hb Bart's hydrops fetalis, at risk couples in whom both partners carry the (—^{SEA}) α-thalassemia mutation should be identified, so that genetic counseling and prenatal diagnosis may be offered.

The carrier rate of β-thalassemia is also high in Southeast Asia. It ranges from 3.4% in Hong Kong[2] to 3-9% in Thailand.[3] Importantly, among β-thalassemia carriers, 4-14% of them are expected to concurrently carry the (--^{SEA}) α-thalassemia mutation. Unless these individuals are correctly diagnosed, antenatal diagnosis for pregnancies at risk of Hb Bart's hydrops fetalis will not be undertaken even if their spouses are (--^{SEA}) α-thalassemia carriers.[4] During the past year, we prospectively screened for the presence of (--^{SEA}) α-globin gene deletion by polymerase chain reaction (PCR)[5] in all β-thalassemia heterozygotes who were diagnosed through a community-based thalassemia screening program in Hong Kong.

A total of 88 individuals were diagnosed to be heterozygous for β-thalassamia mutation by microcytosis and raised Hb A_2 level of > 3.5% on high performance liquid chromatography. The presence of concurrent (--^{SEA}) α-thalassemia was confirmed in eight (9%) individuals (Table 1). Five (cases #1-3, 5, 7) were requests for pre-marital counseling and three (cases #4,6,8) had a positive family history of thalassemia. Their hematologic findings were indistinguishable from simple β-thalassemia heterozygotes except for slightly higher MCV values. Significantly, the partners of two of these individuals (cases #2 and 4) were α^0-thalassemia carriers making these couples at risk of conceiving fetuses with Hb Bart's hydrops fetalis. This information would have been missed and appropriate counseling and prenatal diagnosis would not be offered to these two couples if screening for (--^{SEA}) α-thalassemia had not been undertaken.

If one partner of a couple is a known carrier of α^0-thalassemia, it is imperative to screen for α^0-thalassemia in the other partner even though he/she might appear to be a β-thalassemia carrier based on hematologic findings.[6] In Southeast Asia, where thalassemia is prevalent, we recommend that detection of the co-existing (--^{SEA}) α-thalassemia and β-thalassemia should be undertaken in a community-based thalassemia screening program. This genetic information is of reproductive significance and appropriate counseling can be made available to individuals being screened. Furthermore, this finding is also useful for counseling and diagnosis of other family members. As shown in Table 1, all individuals who are heterozygous carriers of both β-thalassemia mutation and (--^{SEA}) α-thalassemia had microcytosis (MCV less than 80 fL) and elevated Hb A_2 level.[1] However, the Hb H inclusion test was negative in all but one. The screening for concurrent (--SEA) α-thalassemia mutation in these subjects can be done by either embryonic ζ-globin chain assay or by PCR-based techniques.[5,7]

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References

1. Chui DHK, Waye JS. Hydrops fetalis caused by α-thalassemia: an emerging health care problem. Blood 1998; 91:2213-22.
2. Lau YL, Chan LC, Chan YY, et al. Prevalence and genotypes of α- and β-thalassemia carriers in Hong Kong: implications for population screening. N Engl J Med 1997; 336:1298-301.
3. Fucharoen S, Winichagoon P, Thonglairuam V. β-thalassemia associated with α-thalassemia in Thailand. Hemoglobin 1988; 12:581-92.
4. Lam YH, Ghosh A, Tang MHY, Chan V. The risk of α-

thalassaemia in offspring of β-thalassaemia carriers in Hong Kong. Prenatal Diag 1997; 17:733-6.
5. Chan AY, So CK, Chan LC. Comparison of the HbH inclusion test and a PCR test in routine screening for alpha thalassaemia in Hong Kong. J Clin Pathol 1996; 49:411-3.
6. British Committee for Standards in Haematology. Guideline: The laboratory diagnosis of haemoglobinopathies. Br J Haematol 1998; 101:783-92.
7. Lafferty J, Crowther M, Waye JS, Chui DHK. Assessment of a z-globin enzyme-linked immunosorbent assay (ELISA) for the detection of α-thalassemia trait. Blood 1999; 94(Suppl 1):424a.

Relationship of human plasma leptin concentration with blood cell parameters

No strong relationship between leptin and hematologic values was found in a large series of European patients and control subjects. However a role for high levels of leptin in situations such as obesity or sepsis cannot be excluded, as suggested by the weak correlation between leptin and leukocyte count observed in hospitalized patients.

Sir,

Leptin is expressed by adipocytes and involved in the regulation of body weight and body fat. The characterization of a leptin receptor on $CD34^+$ cells[1-3] and a proliferative effect of leptin on hematopoietic cells in culture[1,2,4,5] suggest a relationship between leptin and hematopoiesis. Our demonstration that bone marrow adipocytes secrete leptin supports this hypothesis.[6]

A negative correlation between leptin and hemoglobin in male Japanese adults has been reported, as has a lack of correlation between leptin and leukocytes.[7] These results appear contradictory with the finding of an association between leptin and leukocytes and erythrocytes in Japanese males aged 15-16 years,[8] and with the observation that, in obese Pima Indians, most of the variance in the leukocytes attributable to body fat could be accounted for by leptin concentration.[9] These contradictory findings concerning particular populations (male adolescents in a narrow age range or constitutionally obese Indians) led us to examine the relationship between leptin and blood cell parameters in a large population of European subjects.

Plasma leptin was determined by radio immunoassay (Linco Research Inc, St Charles, MO, USA) in 300 randomly selected hospitalized patients and 70 healthy normal-weight controls (Table 1). Hospitalized patients had a wider range of leptin concentrations (0.07-147 ng/mL) than control subjects (0.1-16 ng/mL), and levels over 20 ng/mL were observed in 8% of patients. In both populations, leptin concentration was significantly higher in women than in men, even after controlling for body mass index (BMI: kg/m^2), so multiple regression analyses were performed to control for gender. In neither population, was BMI correlated with any hematologic parameters. Leptin was weakly correlated ($p< 0.05$) with leukocytes in hospitalized patients. However, a mean comparison of leukocytes between the patients with leptin >20 ng/mL and the other patients was not significant. In controls, we found no correlation between leptin and leukocytes independently of gender or smoking habit. In neither group, was there any correlation between leptin and erythrocytes, hematocrit, hemoglobin, platelets, or any parameter of the differential analysis.

Table 1. Patient characteristics.

	Men	*Women*	*p value*
Hospitalized patients			
N	146	144	
Age (years)	56±17	53±21	
Weight (kg)	72.9±13.5	60.4±9.4	≤0.01
BMI (kg/m^2)	24.3±3.6	22.8±2.6	
Leptin (ng/mL)	3.52±6.12	11.37±19.43	≤0.0001
Leukocytes ($x10^9$/L)	7.81±2.46	7.51±2.56	
Red blood cells ($x10^{12}$/L)	4.39±0.78	4.29±0.57	
Hemoglobin (g/L)	134±24	128±17	≤0.05
Platelets ($x10^9$/L)	257±114	276±104	
Control subjects			
N	28	42	
Age (years)	35±13	31±12	
Weight (kg)	71.9±8.2	55.8±6.2	≤0.0001
BMI (kg/m^2)	22.5± 2.7	20.9±2.0	≤0.01
Leptin (ng/mL)	1.31±1.25	5.95± 3.48	≤0.0001
Leukocytes ($x10^9$/L)	6.48±1.61	6.49±1.48	
Red blood cells ($x10^{12}$/L)	4.90±0.40	4.52 + 0.26	≤0.001
Hemoglobin (g/L)	150±9	138±8	≤0.0001
Platelets ($x10^9$/L)	217±45	245±51	≤0.05

Values are mean±SD. BMI: body mass index.

Although constituting an heteregeneous group, patients had a normal range of BMI, and their mean leptin was comparable to that of controls and that reported for non-obese subjects.[10] High leptin concentrations are reported in various diseases, such as renal failure and sepsis.[10] Leptin secretion is also influenced by a variety of agents, such as dexamethasone, adrenoceptor agonists, and insulin.[10] In this study, no correlation could be established between leptin concentration and clinical status or treatment regimen. However, many patients were suffering from a critical illness. It was reported that leptin was increased in critically ill septic patients, while its circadian rhythmicity was altered.[10] Thus, it can not be excluded that results from some patients were dependent on the moment of blood sampling.

We found no significant relationship between leptin levels and blood cell parameters in healthy midleaged males and females. However, a role for high concentrations of leptin in situations such as obesity or sepsis cannot be excluded, as suggested by the weak correlation we observed in hospitalized patients. In these situations, leptin could contribute, with other cytokines, to the subtle regulation of hematopoiesis.

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