Transferrin receptor-2 polymorphisms and iron overload in transfusion-independent β-thalassemia intermedia

We investigated the relationship between transferrin receptor-2 (TFR2) polymorphisms, namely exon 5 I238M and IVS16 +251 CA deletion, and iron overload in 28 Chinese patients with transfusion-independent β-thalassemia intermedia. There were no significant differences in ferritin level and transferrin saturation between those with or without TFR2 polymorphisms. The number of patients with complications due to iron overload and on desferrioxamine therapy was also not increased among those with TFR2 polymorphisms. Other genetic determinant(s) that may affect the degree of iron overload should be sought in our thalassemia population.

Haematologica 2003;88:345-346
(*http://www.haematologica.org/2003_3/88345.htm*)

A common complication in β-thalassemia major and intermedia is organ damage due to iron overload. While this is attributable to repeated blood transfusions and increased intestinal iron absorption, the co-inheritance of genetic hemochromatosis (GH) alleles may potentially aggravate iron overload. Molecular studies have shown that GH is predominantly caused by a mutation of the HFE gene, with C282Y, H63D and S65C being the more frequently encountered HFE mutant alleles worldwide. Previous studies have shown that co-inheritance of C282Y and H63D alleles affect iron loading in β-thalassemia.[1-5] HFE mutations are nevertheless uncommon among the Chinese population in which β-thalassemia is prevalent. A previous global prevalence study shows that, in Hong Kong Chinese, C282Y is absent and the allelic frequency of H63D is 2.8%.[6] We screened 149 Chinese patients with β-thalassemia major and intermedia for H63D and S65C using a polymerase chain reaction (PCR) restriction analysis based on published protocols.[6,7] Our results showed an absence of S65C mutation and four H63D heterozygotes, giving an allelic frequency of 1.3% for H63D in our cohort of patients (unpublished data). More recently, the gene encoding a second transferrin receptor that mediates cellular iron transport, termed transferrin receptor-2 (TFR2) gene, has been cloned.[8] Mutations of this gene define a new type of GH.[9] These mutations are rare and are mostly found in Italian families. Two polymorphisms of the TFR2 gene, I238M at nucleotide 714 of exon 5 and CA deletion at IVS16 +251, are encountered in Asians.[10] We detected TFR2 polymorphisms I238M and IVS16 +251 CA deletion by PCR amplification of target DNA sequences based on published primers and protocol[10] followed by allele specific oligomer hybridization. Heterozygotes and homozygotes for the polymorphisms were confirmed by automated sequencing of the PCR product (ABI-PRISM 377, Applied Biosystems, Foster City, CA, USA). Heterozygosity for I238M was detected in 20 out of 141 Chinese patients with β-thalassemia major and intermedia, and 11 out of 117 normal controls, giving allelic frequencies of 7.1% and 4.7%, respectively, in the two groups ($p = 0.24$ by the χ^2 test, non-significant). No homozygosity was found. Heterozygosity and homozygosity for IVS16 +251 CA deletion were detected in 57 and 5 out of 137 Chinese patients with β-thalassemia major and intermedia. The corresponding heterozygosity and homozygosity figures for normal controls were 52 and 4 out of 135 subjects. The allelic frequencies were 24.5% among β-thalassemia patients and 22.2% among controls ($p = 0.54$ by χ^2 test, non-significant).

We subsequently investigated the relationship between TFR2 polymorphisms I238M and IVS16 +251 CA deletion with iron overload in 28 Chinese patients with transfusion-independent β-thalassemia intermedia, as the influence of hemochromatosis gene mutations might be masked by severe iron overload in regularly transfused β-thalassemia major.[5] The genotypes in these 28 patients comprised compound heterozygous β^0/β^+ or β^0/β^{++} -thalassemia (n = 8), homozygous nt – 28 (A→G) (n = 5), HbE /β-thalassemia (n = 5), compound heterozygous β^0/β^+ and concurrent ($--^{SEA}$) α-thalassemia deletion (n = 4), heterozygous β^0-thalassemia and triplicated α-globin gene (n = 4), compound heterozygous $\beta^+/\delta\beta$-thalassemia (n = 1) and Hb Malay/β-thalassemia (n = 1). Ferritin was measured by a 2-site sandwich chemiluminometric assay on the ACS:Centaur Chemiluminescence System (Bayer Diagnostics, Tarrytown, NY, USA). Transferrin was measured by rate nephelometry on an Immage Immunochemistry System (Beckman Coulter Instruments Inc., Ireland). Serum iron was measured by a colorimetric method on the Hitachi-912 autoanalyzer (Boehringer-Mannheim, Germany). Transferrin saturation (in %) was calculated by serum iron concentration (mmol/L) × 0.077/transferrin concentration (g/L) × 100%. Serum ferritin and transferrin saturation was calculated as the mean level over the past two years.

There were no significant differences in ferritin level and transferrin saturation between patients with transfusion-independent β-thalassemia intermedia with or without I238M and IVS16 +251 CA deletion (Table 1). The apparently (but not significantly) higher ferritin level in the group without IVS16 +251 CA deletion than in the group with the deletion was most probably attributable to the age difference between the two groups. The nine youngest patients (mean age = 22) without the deletion had a mean ferritin level of 955 μg/L, comparable to the mean ferritin level of the heterozygous group as a whole. Furthermore, the number of patients with compli-

Table 1. Relationship between *TFR2* polymorphisms and iron overload in transfusion-independent β-thalassemia intermedia.

Clinical parameter	*Exon 5 I238M (C→G)*			*IVS16 +251 CA deletion*			*IVS16 +251 CA deletion*
	Normal	*Hetero*	*p value*	*Normal*	*Hetero*	*p value*	*Homozygous*
Number of patients§	23	5	NA	18	9	NA	1
Age (years)	28±3	36±11	0.53†	36±4	19±4	0.02†	21
Hemoglobin (g/dL)	8.3±0.2	7.9±0.2	0.47†	8.0±0.1	8.5±0.4	0.41†	11
Ferritin (mg/L)	1110±226	932±219	0.69†	1305±276	770±153	0.38†	164
Transferrin saturation (%)	93±1.5	91±4.5	0.91†	91±2.0	93±1.8	0.95†	ND
Patients with complications*	4	0	0.57‡	3	1	1.0‡	0
Patients on DFX	6	1	0.40‡	5	2	1.0‡	0

*Key: normal, negative for polymorphism; hetero, heterozygous for polymorphism; NA, not applicable; ND, not done; DFX, desferrioxamine. The range of ferritin values obtained in normal healthy university students is 52 – 398 μg/L for males and 6.8 – 149 μg/L for females. Note: 1) §Included one patient who was negative for I138M and IVS16 +251 CA deletion but heterozygous for H63D mutation of HFE gene. 2) *Four patients had complications attributed to iron overload: Patient #1 (heterozygous for IVS16 +251 CA deletion), liver dysfunction; Patient #2, hypogonadism, atrial fibrillation, congestive heart failure, cirrhosis, diabetes mellitus; Patient #3, hypothyroidism; and Patient #4, hypogonadism. 3) Age, hemoglobin, ferritin and transferrin saturation expressed as mean ± standard error. 4) †:p value by Mann-Whitney test; ‡:p value by Fisher's exact test.*

cations due to iron overload and on desferrioxamine therapy was not increased among those with TFR2 polymorphisms. Our results show that the TFR2 polymorphisms, I238M and IVS16 +251 CA deletion, while prevalent in Chinese patients, do not influence the degree of iron loading in transfusion-independent β-thalassemia intermedia. These TFR2 polymorphisms are therefore not useful in explaining the severe iron overload that may be encountered in our patients. This agrees with findings on I238M polymorphism in normal Asian subjects.[10] Furthermore, detection of common HFE polymorphisms is also not expected to be fruitful, given the low prevalence of these in our area. Nevertheless, the presence of other, hitherto unidentified genetic determinant(s) of iron overload in the Chinese population cannot be excluded and may need to be unraveled in the future.

Edmond S. K. Ma,* Karen K. Y. Lam,* Amy Y. Y. Chan,* Shau-Yin Ha,° Wing-Yan Au,# Li-Chong Chan*

Departments of Pathology, Pediatrics and Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam Road, Hong Kong

Correspondence: Dr. Edmond S. K. Ma, Division of Hematology, Department of Pathology, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong. Phone: international +852.285 4570. Fax: international +852. 28177565. E-mail: eskma@hkucc.hku.hk

Funding: this study was supported by the Children's Thalassemia Foundation and Research Grants Council (HKU 7323/02M). The authors thank Dr. Richard Pang, Division of Clinical Biochemistry, Department of Pathology, Queen Mary Hospital, for performing the iron profile assays.

Key words: TFR2 polymorphism, iron overload, β-thalassemia intermedia, genetic hemochromatosis.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received November 20, 2002; accepted February 6, 2003.

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Molecular characterization of glucose-6-phosphate dehydrogenase deficiency in the Fars province of Iran

We investigated 78 glucose-6-phosphate dehydrogenase (G6PD)-deficient alleles from the Fars province of Iran by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and direct sequencing. The frequency of G6PD Mediterranean in Fars was 84.6%, G6PD Chatham was found to be highly polymorphic and two other sporadic variants (G6PD A- and G6PD Canton) were detected in single cases.

Haematologica 2003;88:346-347
(*http://www.haematologica.org/2003_3/88346.htm*)

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzyme disorder in humans and is characterized by considerable biochemical and molecular heterogeneity.[1] The prevalence of G6PD deficiency in the Middle East varies greatly, ranging from 1% among Egyptians to 11.55% among Iranians.[2,3] G6PD Mediterranean (563 C→T) mutation is probably the most common G6PD variant in the world; it has been widely reported in Europe but also in the Middle-East and in neighboring countries not bordering the Mediterranean sea.[4,5] Among the known variants, the relative frequency of this mutation ranges from 70% among Egyptians to 97% for Kurdish Jews.[6] A recent study carried out on the population of the Mazandaran state of North Iran near the Caspian sea showed a frequency for the G6PD Mediterranean mutation of 66.2% and the presence of two other polymorphic mutations: G6PD Chatham^{1003A} (27%) and G6PD Cosenza^{1376C} (6.7%).[7] We report here a study performed on 78 G6PD-deficient alleles from a different population of Iran, originating from the Fars province, located in the South of the country. The incidence of G6PD deficiency in this area is estimated to be about 12% in males and 0.9% in females.[3]

The study was carried out on 74 unrelated G6PD deficient subjects (66 males, 8 females) aged between 10 days to 20 years (mean 8±5 years) all originating from the Fars province of Iran. The subjects were recruited from neonatal and school screening. The diagnosis of G6PD deficiency was based on the fluorescent spot test. Clinical data were recorded considering neonatal jaundice, favism or drug-related hemolysis.

As preliminary screening, the following polymorphic G6PD molecular variants were tested by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP): G6PD Mediterranean^{563T}, G6PD A-$^{376G/202A}$, G6PD Seattle^{844C}, G6PD Aures^{143C} and G6PD Santamaria$^{376G/542T}$.

The G6PD Mediterranean mutation was detected in 62/74 (83.8%) samples. Four females were homozygous for this mutation, leading to an overall allele frequency of 84.6%. Among the other variants screened for, we identified one subject with G6PD A- (1.3%) whereas G6PD Seattle, G6PD Aures and G6PD Santamaria were absent from all our samples. The 11 negative samples were submitted to SSCP analysis of the entire G6PD coding region that allowed us to identify two different abnormal patterns in exon 9 and 12, respectively. Nucleotide sequencing of exon 9 revealed a G to A substitution at nt 1003 responsible for