The Turkish Journal of Pediatrics 1997; 39: 253-256

COMPOUND HETEROZYGOSITY FOR HEMOGLOBIN KNOSSOS [$\alpha_2\beta_2$ 27 (B9) Ala – Ser] and IVS I – 1 MUTATION*

*Aytemiz Gürgey MD***, *Hatice Balkan BS****, *Gülersu İrken MD*****
*Fatma Gümrük MD******, *Sedat Altay BS*******, *Ali Kalaycıoğlu PhD********
*Cihan Öner PhD*********, *Reyhan Öner PhD*********

SUMMARY: Gürgey A, Balkan H, İrken G, Gümrük F, Altay S, Kalaycıoğlu A, Öner C, Öner R. (Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, and Department of Biology, Faculty of Science, Hacettepe University, Ankara, and Hematology Unit, Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey). Compound heterozygosity for hemoglobin Knossos [$\alpha_2\beta_2$ 27 (B 9) Ala-Ser] and IVS I-1 mutation. Turk J Pediatr 1997; 39: 253-256.

A three-year-old female with compound heterozygosity for Hb Knossos and IVS-I-1 mutation is presented. On physical examination she had no abnormality except for pallor. Hb was 6.9 g/dl, MCV 61 fl, Hb A2 2% and Hb F 38.5%. Acrylamidegel electrophoresis at a pH of 6 revealed the presence of Hb Knossos in the child and her father. DNA studies revealed that the child was compound heterozygous for Hb Knossos and the IVS I-1 mutation. When the clinical expression of this combination in a previously reported patient with Hb Knossos/FSC8 mutation is compared, it is shown that the newly presented patient has a more severe condition, indicating that the mutations in the trans of Hb Knossos may play a role in the phenotypical expression of the disease.
Key words: Hb Knossos, β-thalassemia, mutation, abnormal hemoglobin.

Hemoglobin (Hb) Knossos (β-27 Ala-Ser) results from a mis-sense mutation at codon 27 which causes abnormal RNA processing[1,2]. Although Hb Knossos cannot be shown in routine electrophoretic study, abnormal β-chain can easily be detected by acrylamide gel electrophoresis[1-4]. Hb Knossos was originally reported in a Greek patient with β-thalassemia intermedia[1]. Later it was found in patients from Algeria and Egypt[3,4] and in Turkey in a patient with trans of frame shift codon 8 (FSC8) mutation whose clinical and hematological picture was very mild[5]. Since FSC8 itself is associated with β-thalassemia intermedia,

* From the Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, and the Department of Biology, Faculty of Science, Hacettepe University, Ankara, and the Hematology Unit, Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir.

** Professor of Pediatrics, Hacettepe University Faculty of Medicine.

*** Research Fellow in Molecular Biology, Hacettepe University Faculty of Science.

**** Professor of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir.

***** Associate Professor of Pediatrics, Hacettepe University Faculty of Medicine.

****** Chemist, Hacettepe University Faculty of Medicine.

******* Associate Professor of Molecular Biology, Hacettepe University Faculty of Science.

it was not clear to us whether the mildness of the diseases was due to Hb Knossos mutation or FSC8, or both. Therefore we believe that comparison of this patient with a patient with compound heterozygosity for Hb Knossos and a severe β-thalassemia mutation would be interesting.

Case Report

A three-year-old girl was referred to Dokuz Eylül University for evaluation of anemia. She was the first product of a nonconsanguineous marriage and had been pale since one year of age. One year previous to her referral, she received transfusion of one unit of blood.

On physical examination at admission, her weight was 14 kg, height 95 cm (both were at the 50th percentile) and there was no organomegaly. In laboratory studies, Hb was 6.9 g/dl, packed red cells 0.19 L/L, MCV 61 fl, Hb A_2 2 percent and Hb F 38.5 percent. The Hb A_2 and Hb F levels of the mother and father are shown in Table I. The presence of a slient mutation was predicted due to the normal Hb A_2 level in the father. Therefore, acrylamide gel electrophoresis was performed, and the presence of Hb Knossos mutation. was detected in the patient and her father. DNA was extracted from the patient's white blood cells and from both parents, according to the procedure described by Poncz et al.[6] The presence of α-thalassemia was studied by digestion of genomic DNA with Bam HI and Bgl II and hybridization to the α-specific gene probe, as described previously[7].

Table I: Hematologic Data of The Family

	Age (Years) and Sex*	Hb g/dl	MCV fl	HbA2 %	HbF %	Genotype
Propositus	3F	6.9	61	2.0	38.5	Hb^{Knos}/IVS-1-1
Mother		11.0	56	4.9	1.7	IVS-1-1/β^A
Father		13.0	72	2.5	0.9	Hb^{Knos}/β^A
Case 2	17M	8.0	77	1.2	46.5	Hb^{Knos}/FSC8 (ref: 5)

* Age at presentation.

In-vitro amplification of the β-globin gene region was performed by two-stage polymerase chain reaction (PCR) described by Saiki et al.[10] with minor modifications using a DNA Thermal Cycler. For the first-stage amplification, a 1860 bp double-stranded fragment of β-globin region was obtained using equal amounts of the primers (100 pmol) at position -158 in the promoter region and at position 128 after the poly A signal in the 3' untranslated region. For the second-stage amplification, the region between position -148 in the promoter region and position 147 in the IVS II region of the β-globin gene was asymmetrically amplified using the 5' primer as the limiting one. Direct sequencing of

asymmetrically amplified DNA was done using the dideoxy terminating method of Sanger[8]. Sequence analysis indicated the presence of compound heterozygosity for Hb Knossos and IVSI-1 G-T mutation in the patient (Fig. 1). The father was heterozygous for Hb Knossos and the mother for IVS I-1.

Discussion

Hb Knossos is one of the hemoglobinopathies that causes β-thalassemia intermedia such as the clinical and hematological picture in homozygous and

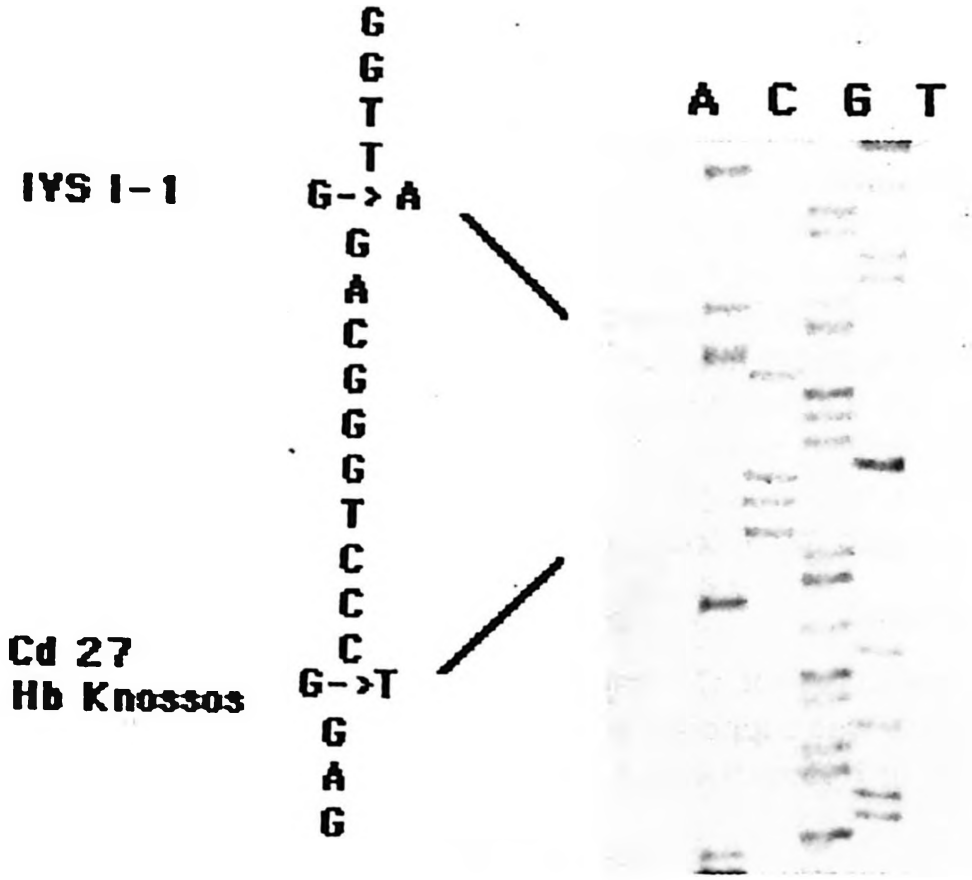

Fig. 1: Sequence analysis for Hb Knossos and IVS-I-I G-T mutation.

compound heterozygous (a β-thalassemia mutation in trans) conditions. Although Hb F slightly increases in homozygous conditions, there is no increase in Hb F and Hb A_2 values in Hb Knossos traits, though an appreciable increase in the Hb F value is shown in compound heterozygotes who have a β-thalassemia-intermedia-like clinical picture. Red-cell indices and the Hb A_2 values of the parents would be useful for detecting Hb Knossos traits that usually have mild microcytosis associated with normal Hb A_2 values[2]. On the contrary, the -101 mutation of the β promotor region and a normal Hb A_2 value are associated with normal red cell indices[9]. Absence of an elevation in Hb A_2 value in Hb

Knossos homozygotes was explained on the basis of a second mutation resulting in δ^o-thalasemia by Baklouti et al.[4] However, a similar mutation could not be found in other subjects with Hb Knossos. Mutations in codon 26 (Hb E) and codon 27 (Hb Knossos) were accepted as thalassemic hemoglobinopathies because they create an abnormality in the processing mechanism[2].

The molecular basis of the β-thalassemia mutations in the trans of Hb Knossos was unknown in the majority of the previously published patients who were compound heterozygous for Hb Knossos and β-thalassemia[1, 3, 4, 10]. Therefore, the effect of β-thalassemia mutations in the trans of Hb Knossos on the clinical and hematological expression of the disease is unknown. Our previously pusblished patients had FSC8 mutation, and the present patient had IVS 1-1 mutation[5]. Although both of these mutations result in β^o-thalassemia, the first one was regarded as one of the well-studied mild β^o-thalassemia mutations. Indeed when the hematological parameters of these two patients were compared, it seemed that the condition associated with Hb Knossos/FSC8 mutation was milder than Hb Knossos/IVSI-1 combination (Table I). However the latter condition could still be regarded as β-thalassemia intermedia (Table I). This indicates that Hb Knossos itself is a very mild thalassemic mutation. We believe that inclusion of acrylamide gel electrophoresis in hematological studies in thalassemia intermedia patients with normal Hb F and Hb A_2 values and heterozygotes with mild microcytosis and normal Hb A_2 values will increase the detection of subjects with the Hb Knossos mutation.

Acknowledgement

We are very grateful to Prof. Ç. Altay of Hacettepe University for her continuous support. This study was supported by TUBITAK (Turkish Scientific and Technical Research Council) Reseach Grant Tag-1218.

REFERENCES

1. Fessas P, Loukopoulos D, Loutradi-Anagnostou A, Komis G. 'Silent' beta-thalassaemia caused by a 'silent' beta-chain mutant: the pathogenesis of a syndrome of thalassaemia intermida. Br J Haematol 1982; 51: 577-583.
2. Orkin SH, Antonarakis SE, Loukopoulos D. Abnormal processing of $beta^{Knossos}$ RNA. Blood 1984; 64: 311-313.
3. Elwan S, Baklouti F, el-Kabsh M, Abdelrahman F, Delaunay J. Hemoglobin Knossos [$alpha_2$ beta 2 (27) (B9) Ala-Ser] in Egypt. Hemoglobin 1987; 11: 139-143.
4. Baklouti F, Dorleac E, Morle' L, et al. Homozygous hemoglobin Knossos (alpha 2 beta 2 27 (B9) Ala-Ser): a new variety of beta thalassemia intermedia associated with delta degree-thalassemia. Blood 1986; 67: 957-961.
5. Kutlar A, Kutlar F, Aksoy M, et al. Beta-thalassemia intermedia in two Turkish families is caused by the interaction of Hb Knossos [beta 27 (B9) Ala-Ser) and of Hb City of Hope [beta69 (E13) Gly-Ser] with beta degree-thalassemia. Hemoglobin 1989; 13: 7-16.

6. Poncz M, Solowiejczyk D, Harpel B, Mory Y, Schwartz E, Surrey S. Construction of human gene libraries from small amounts of peripheral blood: analysis of beta-like globin genes. Hemoglobin 1982; 6: 27-36.
7. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975; 98: 503-517.
8. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 1977; 74: 5463-5469.
9. Gonzalez-Redondo JM, Stoming TA, Kutlar A, et al. AC-T substitution at nt-101 in a conserved DNA sequence of the promoter region of the beta-globin gene is associated with 'silent' beta-thalassemia. Blood 1989; 73: 1705-1711.
10. Fessas P, Loukopoulos D, Kokkinou S, Papasotiriou Y, Karaklis A. Hemoglobin Knossos: a clinical, laboratory, and epidemiological study. Am J Hematol 1986; 21: 119-133.