Compound heterozygosity for two beta chain variants: the mildly unstable Hb Tyne (codon 5 Pro→Ser) and HbS (codon 6 Glu→Val)

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ABSTRACT

Compound heterozygosity for Hb Tyne and HbS, that is very rare, was identified by direct DNA sequencing of the beta-globin gene in a Turkish patient. Hematological investigation of a girl at the age of 9 due to the presence of HbS (40.7%) led to the identification of a compound heterozygosity at codons 5-6. This was found to be the result of substitution of cytosine (C) for thymidine (T) at the fifth position and a substitution of adenine (A) for thymidine (T) at the sixth position of the beta globin gene. As a result of these mutations, the order of amino acids at codons 5-6 was changed from Pro-Glu to Ser-Val, respectively. Since the co-inheritance of Hb Tyne and HbS had not been reported in literature before, our case set an example for identification of co-inheritance of Hb Tyne and HbS for the first time. Therefore, such cases may be considered as an important example for understanding the structural variants of hemoglobin and may provide important clues for critical amino acids responsible for stabilization of hemoglobin tetrameric structure and genetic counseling.

Key Words: Hemoglobin S, Hemoglobin Tyne, Beta-globin, Heterozygosity, DNA sequence.
INTRODUCTION

Hemoglobinopathies are known as the most common single gene defects in the world population. About 5% of world’s population are carriers of different inherited hemoglobin (Hb) and thalassemia disorders, and more than 300,000 severely affected homozygous or compound heterozygous are born each year[1]. Different structural hemoglobin variants have been reported using hemoglobin electrophoresis and molecular techniques. While about half of these variant are clinically silent, other half that are not silent generally generate a change in oxygen affinity or a physically unstable Hb molecule[1-4]. Sickle cell hemoglobin (HbS) that is the most common Hb variant was firstly identified as abnormal hemoglobin and has great clinical importance because of causing hemolytic anemia. In HbS, a single glutamic acid residue on beta-globin chain or chains is replaced by valine residue (codon 6 Glu → Val). This single amino acid change results in decreased ability of globin to transport oxygen. Homozygous, heterozygous, beta-thalassemia/sickle cell, or other compound heterozygous are the phenotypic consequences of the mode of inheritance[5-7]. The unstable hemoglobin disorders result from the presence of structurally abnormal hemoglobin variant with substitution or deletion of amino acid in globin chains[8]. Unstable hemoglobin variants are either degraded before the tetramer assembly or immediately after it is made in the bone marrow[9]. The unhybridizable octamer [(g) 14] is the result of the presence of an additional amino acid in the tetramer[10]. The other unstable hemoglobin variants have been illustrated. These hyper unstable variants offer important clues to identify structurally critical areas of the hemoglobin tetramer[10]. Langdown JV et al firstly discovered Hb Tyne in two unrelated English citizens in 1994. Hb Tyne (codon 5 C → T) results from a single amino acid change in beta-globin polypeptide: a substitution of proline for serine at the fifth position of the beta-globin polypeptide (codon 5 Pro → Ser). They hypothesized that this mutation causes formation of mildly unstable hemoglobin[11]. We know that the co-inheritance of two Hb variant in a patient is very uncommon. Here, we are reporting the co-inheritance of Hb Tyne (codon 5 Pro → Ser) and HbS (codon 6 Glu → Val) in a 9-years-old girl diagnosed clinically with beta-thalassemia/HbS for the first time.

A CASE REPORT

A 9-years-old girl was referred to Thalassemia Center, Antalya State Hospital because of soft skin, anemia, weakness, and lack of appetite, two years ago. After clinical examination and HbS, HbF, HbA2 values were determined by HPLC, then she was diagnosed as sickle cell anemia and beta-thalassemia due to increased level of HbS, HbF, HbA2. In physical examination: weight: 14 kg, height: 102 cm, liver: 2 cm, spleen: 4 cm, and blood count showed the following values: RBC: 2.11 x 1012/L, Hb: 6.6 g/dL, MCV: 73.1 fL, MCH: 31.5 pg, HbA1c: 42.5%, HbA2: 3.9%, HbF: 6.1%, HbS: 40.7%. Blood transfusion was annually 7-8 units.

DNA Analysis

DNA was extracted from whole blood using salting-out method[12]. Polymerase chain reaction (PCR) was performed by specific primers of beta-globin gene and amplified DNA was analyzed to investigation known common mutations in Mediterranean Region using β-Globin Strip Assay kit (Vienna Lab) based on reverse dot blot hybridization (RDBH). To confirm the presence of HbS allele DdeI restriction enzyme analysis was performed. To explore the other mutations in beta-globin gene, DNA including...
approximately 700 bp was amplified using amplification primers (Primer F: 5’- GCCAAGGACAGGTACGGCTGTCAT C-3’ and Primer R: 5’-CCCTTCCTATGACATGACTTAACCAT-3’) designed for the initial site of the beta-globin gene. PCR product was separated in a 1.5% agarose gel. The amplified fragment was eluted from agarose gel using purification kit (MBI Fermantas, K0513, Iontek, Bursa, Turkey). Chain termination sequencing with ddNTPs and [α-35S]-dATP was performed on single-stranded PCR templates[13].

RESULTS

The A → T substitution at codon 6 was screened by RDBH and confirmed by DdeI restriction enzyme analysis. DNA sequencing of the patient’s beta-globin gene demonstrated two substitutions in codon 5 and 6: both a substitution of cytosine (C) for thymidine (T) at the fifth position and a substitution of adenine (A) for thymidine (T) at the sixth position of the beta-globin gene (Figure 1). Due to these mutations normal amino acids (Pro-Glu) in beta globin polypeptide were changed to (Ser–Val), respectively according to the following schema:

Normal allele codons: ACT C T G G
Mutant allele codons: ACT T T G G

Normal amino acids: Thr Pro Glu
Mutant amino acids: Thr Ser Val Glu

After two years, blood count of the proband changed the following values: RBC: 2.5 x 1012/L, Hb: 11.0 g/dL, MCV: 94.4 fL, MCH: 30.70 pg, MCHC: 35.20 g/dL, RDW: 16.90%, HbA1: 45.0%, HbA2: 4.4%, HbF: 26.5%, Hbs: 24.5%. Blood transfusion was annually decreased from 7-8 units to 2-3. In addition, serum electrolite levels were found as the following values: Na: 141.00 mEq/L, K: 3.91 mEq/L, Cl: 104.00 mEq/L, Ca: 9.50 mg/dL, and glucose: 88.00 mg/dL, creatinine: 0.24 mg/dL, serum albumin: 3.70 g/dL.

Proband’s father was found as a carrier for Hb Tyne, and mother for HbS. The hematological parameters of father and mother were HbA1 (52%), HbA (4.4%), HbF (1.0%), HbS (36.7%), Hb: 15.6 g/dL, Hct: 46.5%, MCV: 85.4 fL, MCH: 28.7 pg, MCHC: 33.5 g/dL, RDW: 15.1%, and HbA1 (46.7%), HbA2 (5.0%), Hbf (0.3%), HbS (48.0%), Hb: 13.9 g/dL, Hct: 40.8%, MCV: 93.2 fL, MCH: 31.7 pg, MCHC: 34.1 g/dL, RDW: 13.7%, respectively. DNA sequencing of the patient’s beta-globin gene is shown in Figure 1.

DISCUSSION

Hereditary disorders that result in structurally abnormal hemoglobin or an insufficient common quantity of Hb are the most common human genetic diseases. Most mutations in the globin genes are a single base pair change in DNA code resulting in amino acid substitutions[1]. Sickle cell anemia is caused by a GAG substitution in codon 6 of beta-subunit of hemoglobin that produces a
structural variant of Hb, HbS. Due to the historical migration of different ethnic groups, HbS does not show homogeneously distribution in Turkey, and generally is common in South-eastern coasts, and Mediterranean region\[14\]. While South-eastern coasts, known as Çukurova region in where the disease frequency varies from 0.3 to 37%\[10\]. The incidence of HbS was found to have 10.3% in Antalya\[15\]. Although the co-inheritance of two Hb variant in a patient is very uncommon, the presence of the patient’s co-inheritance sickle cell anemia with another globin gene disorders such as beta-thalassemia, other hemoglobin variants due to this relatively high incidence of HbS in Antalya population is not surprising. The unstable hemoglobin disorders result from the presence of structurally abnormal hemoglobin variant with substitution or deletion of amino acid in globin chains\[1,4\]. Langdown et al first discovered Hb Tyne in two unrelated English citizens. Hb Tyne (codon 5 C → T) has previously been characterized as mildly unstable hemoglobin variant\[11\]. In this study, we firstly describe a Turkish patient who carried HbS at codon 6 and hemoglobin Tyne codon 5. After HbS and HbF level were shown to be high using electrophoresis, genotype of the patient was found to be a compound heterozygote for HbS and Hb Tyne by molecular studies. The hydroxyurea (HU) (20 mg/kg/day) had been used to decrease the clinical severity for last two years of her life, and blood transfusion necessity of the proband was decreased from 7-8 to 2-3 units, annually. We observed a very good clinical response to HU treatment, and HbF was increased from 6.1% to 26.5% during two years. Also, MCV and Hb levels were found to be increased, 83.4 fL versus 94.4 fL and 8.3 g/dL versus 9.4 g/dL, respectively.

As a conclusion, this report of the co-inheritance of HbS and Hb Tyne may be very important to understand molecular mechanisms of globin gene and may assist to physicians for management of treatment strategies and genetic counseling.

**REFERENCES**


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