Hb G Makassar (Beta 6 : Glu→Ala) in a Thai Family

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Abstract

Hemoglobin G Makassar was identified in a family of Thai origin, which was found to share identical properties with hemoglobin S in routine hemoglobin separation both by cellulose acetate electrophoresis and cation–exchange HPLC. It is, therefore, subjects with hemoglobin G Makassar and hemoglobin S who may sometimes be mistakenly identified for each other. In this study, identification of hemoglobin G Makassar was achieved by DNA sequence analysis, which revealed a single nucleotide substitution GAG→GCG at codon 6 of the beta-globin gene. The hemoglobin variant was hemoglobin G Makassar [beta 6 : Glu→Ala]. This is the first report of hemoglobin G Makassar in Thailand.

Key word : Hemoglobin G Makassar, Thailand

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Hemoglobin variant, a group of autosomal-recessive inherited abnormalities in the structure of hemoglobin is one of the most common genetic defects in Thailand. The gene abnormalities may be due to substitution of one amino acid for another, deletion of a portion of amino acid sequence, abnormal hybridization between two chains or abnormal elongation of the globin chain[1,2]. The abnormal chains may be alpha chain, beta chain, gamma chain or delta chain. To date approximately 750 hemo-

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globin variants have been reported[3], causing a variety of clinical manifestations. Most of them are not clinically apparent. Others produce asymptomatic abnormal hematological laboratory findings. A very few produce serious disease. In Thailand, many hemoglobin variants have been reported such as hemoglobin E[4], hemoglobin Thai (Constant Spring) [5], hemoglobin Tak[6], hemoglobin-J Bangkok[7], hemoglobin Siriraj[8], hemoglobin Q[8], hemoglobin Mahidol[9], hemoglobin New York[10], hemoglobin Anantharaj[11], hemoglobin D-Punjab[12], hemoglobin Thailand[13], hemoglobin Suan-Dok[14] and hemoglobin C[15]. Hemoglobin E and hemoglobin Constant Spring are the two most common hemoglobin variants found in Thailand[16].

Presented in this article is the identification of hemoglobin G Makassar in a family of Thai origin in Songkhla province. Although the structural change of hemoglobin G Makassar (beta 6 : Glu→Ala)[17] occurs at the same position as that in hemoglobin S, their clinical manifestations are absolutely different. Homozygous expression of hemoglobin S produces sickle cell disease, which is a chronic hemolytic anemia and vaso-occlusive condition that usually takes the life of the patient. On the other hand, the subject with homozygous hemoglobin G Makassar found in this study was healthy.

MATERIAL AND METHOD

Hematological analysis

Peripheral blood samples were collected using Na_2EDTA as the anticoagulant. Hemoglobin concentration, erythrocyte indices and other hematological parameters were obtained using a Coulter electronic counter. Screening for thalassemias and hemoglobinopathies were performed using the osmotic fragility test and dichlorophenol indophenol precipitation test. Erythrocyte morphology was examined using standard procedures. Hemoglobin electrophoresis on cellulose acetate and hemoglobin separation and determination by HPLC (Variant, Bio-Rad Laboratories, Hercules, CA, USA) were performed.

Polymerase chain reaction and direct DNA sequencing

DNA was extracted from peripheral blood leukocytes. The beta globin gene fragments were amplified using polymerase chain reaction with designated primers[18]. Direct DNA sequencing was performed with various internal primers using the Sanger's sequencing method[18] and then automatically analyzed by ABI Prism 310 genetic analyzer (Applied Biosystems, Foster city, CA, USA).

RESULTS

According to the prevention and control program for thalassemias and hemoglobinopathies at Songkhla Hospital, blood samples of pregnant women who attended the antenatal clinic are collected and screened by using the osmotic fragility test and dichlorophenol indophenol precipitation test. In this study, the hemoglobin variant was found in a 27-year-old pregnant woman, without any abnormal clinical feature. Results of the osmotic fragility test and dichlorophenol indophenol precipitation test were positive and negative, respectively. The hematological parameters showed a hemoglobin concentration of 13.8 g/dl, hematocrit 39 per cent, red blood cell 3.98 x $10^6/\mu l$, white blood cell 5.58 x $10^3/\mu l$ and platelet 176 x $10^3/\mu l$. The mean corpuscular volume (MCV) was 96.6 fl, the mean corpuscular hemoglobin (MCH) 34.7 pg and the mean corpuscular hemoglobin concentration (MCHC) 35.9 g/dl. The morphology of red blood cell was normal. Results of hemoglobin separation and determination by using HPLC indicated that the retention time (RT) of the hemoglobin variant was 4.56 minutes (S-window) and constituted 93.2 per cent of the whole hemoglobin (Fig. 1). The mobility of the hemoglobin variant in cellulose acetate electrophoresis was identical to that of hemoglobin S.

DNA was extracted from peripheral blood and exon I of the beta globin gene was directly sequenced. A GAG→GCG mutation at codon 6 which leads to the amino acid substition Glu→Ala was identified. The representative chromatograms and DNA sequences of sequencing results are shown in Fig. 2. The hemoglobin variant was hemoglobin G Makassar. Genotype of this family of Thai origin is shown in Fig. 3. Both homozygotes and heterozygotes did not have any abnormal clinical features.

DISCUSSION

A hemoglobin variant was detected in a 27 year-old woman during pregnancy at Songkhla Hospital in the southern part of Thailand where beta globin gene mutations are very heterogeneous and

ANALYTE ID	%	TIME	AREA
F	0.8	1.03	14634
Unknown 1	0.5	1.21	9749
P3	0.3	1.59	6165
AO	4.2	2.14	89171
A2	0.7	3.50	11641
Unknown 2	0.5	3.73	10650
S-WINDOW	93.2	4.56	1977153
	TOTAL AREA		2119163
F	0.8%	A2	0.7%

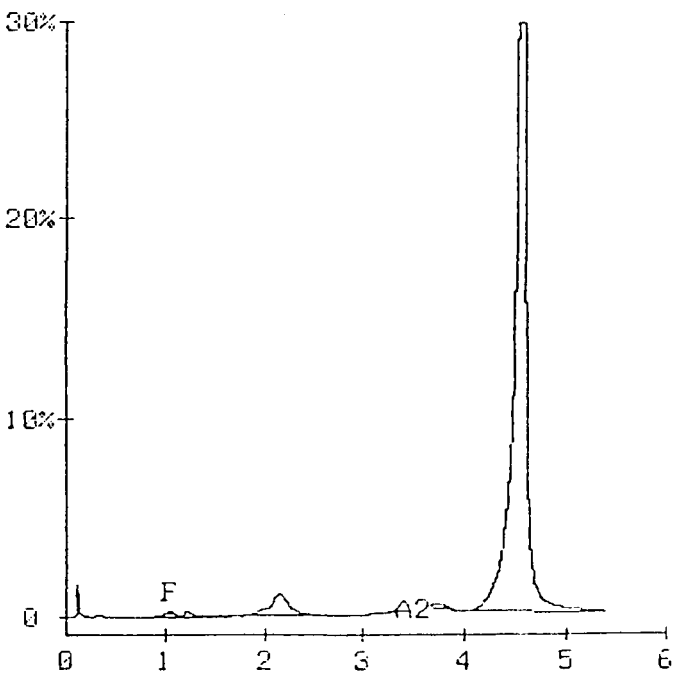

Fig. 1. Cation exchange HPLC chromatogram of hemoglobin G Makassar which has chromatographic properties identical to those of hemoglobin S.

different from those in other regions[19,20]. A hemoglobin variant was found to have the same properties as hemoglobin S in routine hemoglobin separation by cellulose acetate electrophoresis and cation-exchange HPLC. Due to the absense report of hemoglobin S in the Thai population and the discrepancy between the biological data and the clinical presentation in the subject not at risk of sickle cell disease, DNA sequencing was performed to identify the hemoglobin variant. A GAG→GCG mutation at codon 6 which leads to the replacement of glutamic acid by alanine was identified. The subject was found to be homozygous hemoglobin G Makassar. It is not surprising that hemoglobin G Makassar which concerns the same site as hemoglobin S behave similarly in routine hemoglobin analysis both by using cellulose acetate electrophoresis and cation-exchange HPLC. Therefore, hemoglobin G Makassar

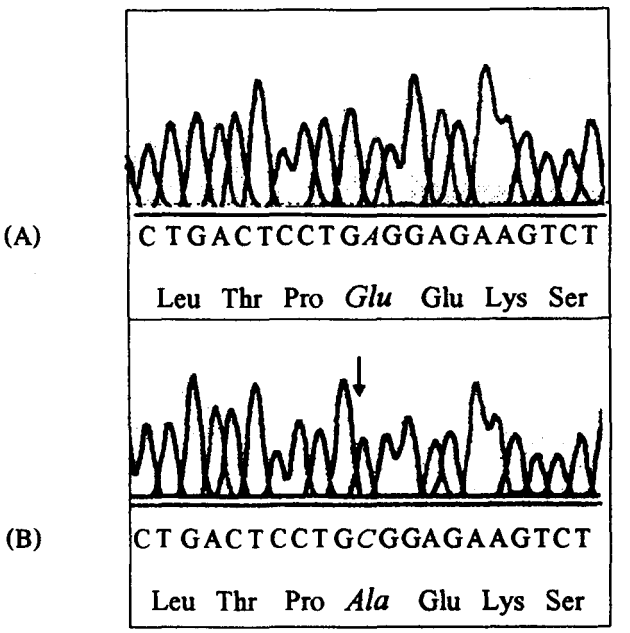

Fig. 2. Chromatograms and DNA sequences of normal (A) and mutant beta globin gene at codon 6 (B). An adenine was substituted by cytosine resulting in the replacement of the normal glutamic acid (Glu) by alanine (Ala).

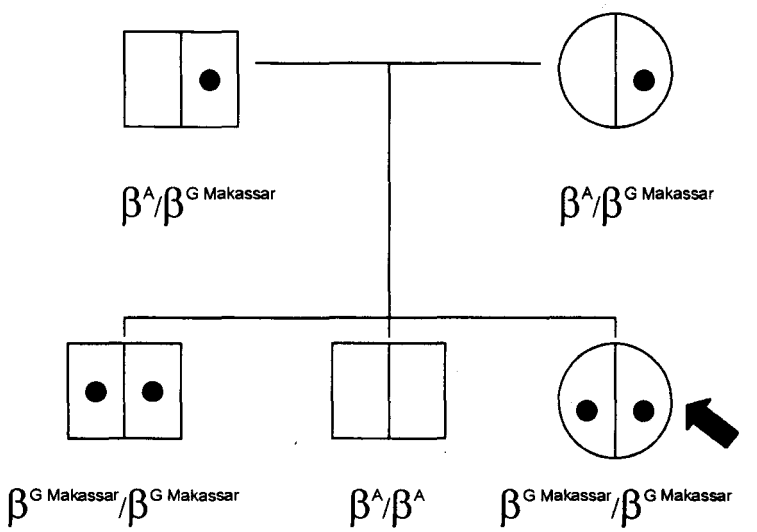

Fig. 3. Pedegree. The 27 year-old pregnant woman is indicated by the arrow.

and hemoglobin S might be mistakenly identified for each other based on which ever variant is most prevalent in the particular population.

Although the structural change of hemoglobin G Makassar occurs at the same position as that in hemoglobin S, their clinical manifestations are absolutely different. Under conditions of low oxygen tension, hemoglobin S polymerize, causing the red blood cells to assume a "sickle shape" leads to the symptoms of sickle cell disease(21). Patients with homozygous hemoglobin S have serious disease concerning a chronic hemolytic anemia and vaso-occlusive condition. On the other hand, the subject with homozygous for hemoglobin G Makassar found in this study did not have any abnormal clinical feature. The difference in clinical manifestations of these two hemoglobin variants might be due to the fact that hemoglobin G Makassar does not polymerize(22).

Hemoglobin G Makassar, previously described in Indonesia(17) was first detected in Thailand by this study. Determination of a haplotype of this mutation should provide useful information about the origin and migration of this gene in the country. However, throughout history, the Indian subcontinent had political, military and commercial interactions with the people of South-East Asia including Malaysia and Indonesia(23). This may reflect the gene flow of this hemoglobin variant to the southern part of Thailand due to people migration over the border between the south of Thailand and Malaysia.

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ฮีโมโกลบิน G Makassar (Beta 6 : Glu→Ala) ในครอบครัวคนไทย

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คณะผู้วิจัยได้รายงานการตรวจพบฮีโมโกลบินผิดปกติชนิด G Makassar ในครอบครัวคนไทย โดยฮีโมโกลบินผิดปกติดังกล่าวไม่สามารถวินิจฉัยแยกจากฮีโมโกลบิน S ได้โดยการตรวจวิเคราะห์ชนิดฮีโมโกลบินในงานบริการประจำวัน ทั้งโดยวิธี cation-exchange HPLC และ cellulose acetate electrophoresis ดังนั้นจึงอาจทำให้การแปลผลผิดพลาดได้ การศึกษาครั้งนี้ได้ทำการตรวจวินิจฉัยชนิดของฮีโมโกลบินผิดปกติโดยการตรวจหาลำดับเบส ซึ่งพบการเปลี่ยนแปลงชนิดของเบสที่ตำแหน่ง codon ที่ 6 จาก GAG เป็น GCG ส่งผลให้มีการเปลี่ยนแปลงของกรดอะมิโนจาก glutamic acid เป็น alanine ซึ่งเป็นฮีโมโกลบินผิดปกติชนิด G Makassar [beta 6 : Glu→Ala] นับเป็นการรายงานการตรวจพบฮีโมโกลบินชนิดนี้เป็นครั้งแรกในประเทศไทย

คำสำคัญ : ฮีโมโกลบิน G Makassar, ประเทศไทย

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