Allele Related Mutation Specific-Polymerase Chain Reaction for Rapid Diagnosis of Hb New York (Beta 113 (G15) Val→Glu, $\beta^{CD113\ GTG\rightarrow GAG}$)

VIP VIPRAKASIT, M.D.*,**, KALAYA TACHAVANICH, M.Sc.**,
LERLUGSN SUWANTOL, B.Sc.**, PARICHAT PUNG-AMRITT, B.Sc.**,
WORAWUT CHINCHANG, B.Sc.**, VORAVARN S. TANPHAICHITR, M.D., M.S.**

Abstract

Hemoglobin New York (beta 113 (G15) Val→Glu), a β-globin variant, was first reported in a Chinese family living in New York. Subsequently, this abnormal hemoglobin was reported in many Chinese descendants from several groups and it was also known as Hb Kaohsiung. The subtle change in $\alpha_1\beta_1$ contact region apart from the heme group connecting area by Val→Glu substitution has minor changes in both the electrophoretic mobility and stability making this hemoglobin variant difficult to distinguish from Hb A using routine hemoglobin analysis. The authors described a case of heterozygosity of Hb New York diagnosed by a molecular technique and revealed a mutation in $\beta^{CD113\ GTG\rightarrow GAG}$. A novel Allele Related Mutation Specific-Polymerase Chain Reaction (ARMS-PCR) for rapid diagnosis of this mutation has been proposed.

Key word : Hb New York, β-Globin Variant, Hemoglobinopathy

VIPRAKASIT V, TACHAVANICH K, SUWANTOL L, PUNG-AMRITT P, CHINCHANG W, TANPHAICHITR VS
J Med Assoc Thai 2002; 85 (Suppl 2): S558-S563

Thalassemia and hemoglobinopathies are very common inherited disorders and have been found in nearly every region throughout the world (1). In particular, Southeast Asia and Southern China where both types of hemoglobin disorder are extremely common, it was estimated that nearly 30 per cent of the population in this area carry one of these disorders(2). Moreover, a wide variety of muta-

* MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, UK.
** Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

tions make up more than 60 different thalassemia and hemoglobinopathies syndromes contributing to the complexity in diagnosis and proper management. Standard hemoglobin studies using electrophoresis or chromatography, sometimes, can easily detect abnormal hemoglobin presenting in red blood cells. However, it is not always the case since some abnormal hemoglobin variants may be hard to detect using standard methods and this increases the difficulty in the diagnosis. The authors reported a case of a healthy woman with Hb New York (beta 113 (G15) Val→Glu) in whom routine hematological and hemoglobin analyses were not able to demonstrate this β-globin variant. To our knowledge, this is the second report of this hemoglobin variant in Thailand. The molecular characterization of mutation responsible for Hb New York (βCD113 GTG→GAG) has been described including a novel Allele Related Mutation Specific-Polymerase Chain Reaction (ARMS-PCR) for rapid diagnosis of this mutation.

CASE HISTORY

A 31-year old healthy woman was referred to the Department of Pediatrics, Faculty of Medicine, Siriraj Hospital for definitive diagnosis of her abnormal hemoglobin. This was accidentally revealed during her laboratory evaluation due to her infertility problem after several years of marriage without gestation. She had no history of anemia, jaundice, blood loss, chronic disease and thalassemia in her family. Her father had migrated from main land China and her mother is of half Thai-Mon descent. Physical examination was unremarkable. Complete blood count was performed and revealed normal hematological profiles 5.04 x 10^{12}/L of red cells count (RBC), 13.9 g/dL of hemoglobin (Hb), 42.6 per cent of hematocrit (Hct), 84.6 fL of mean cell volume (MCV), 27.6 pg of mean cell hemoglobin (MCH), 32.6 g/dL of mean cell hemoglobin concentration (MCHC), 14 per cent of red cell distribution width (RDW), 4 per cent of reticulocytes count and normal platelet and white blood cells counts (WBC). The brilliant cresyl blue staining of peripheral blood for inclusion bodies was negative. Hemoglobin analysis using cellulose acetate electrophoresis in Supre-Heme Buffer (Helena Laboratories, Texas, USA), demonstrated abnormal hemoglobin band (Hb X) running close to Hb A. The proportion of Hb X, Hb A and Hb A2 were 43.3 per cent, 51.8 per cent and 4.9 per cent respectively[3]. However, this abnormal hemoglobin was less distinctive and hardly discriminated from Hb A using the starch gel electrophoresis[4] nor by Low Pressure Liquid Chromatography (LPLC, Hb Gold, Hemoglobin Analyser, Drew Scientific Ltd., UK). To confirm the presence of this Hb X, an isoelectric focusing technique was carried out[5], and this abnormal hemoglobin was clearly demonstrated. This abnormal band ran to the position next to Hb A (Fig. 1) and the quantity was nearly 50 per cent of the total hemoglobin. The presence of approximately 45-50 per cent of abnormal hemoglobin in this woman suggests that this abnormal hemoglobin should result from the β-globin chain variant and prompted the authors to evaluate the molecular defect of the structure of β-globin gene in which Hb New York is suspected.

MATERIAL AND METHOD

Genomic DNA was extracted from peripheral leucocytes using EDTA as an anticoagulant using the method described elsewhere[6]. To detect the mutation, 2.4 kb region of the β-globin genes extending from -124 bp upstream of the cap site to +163 downstream of the poly A site were amplified by a polymerase chain reaction (PCR), and mutations were identified by direct nucleotide sequencing of the PCR product using fluorescent labeled dideoxy-terminator (Perkin Elmer Biosystems Co., Norwalk, USA) and analyzed on an ABI 377-automated sequencer (Applied Biosystems, Foster City, USA).

To confirm the mutation found in Hb New York (βCD113 GTG→GAG), the authors designed an Allele Related Mutation Specific-Polymerase Chain Reaction (ARMS-PCR). The common forward primer (F1); 5′-CAATGTATCATGC-CTCTTTGC ACCATT-3′ was synthesized and used with either one of two reverse primers (one is specific for amplification of the normal allele; Rn; 5′-GCCAAAGT GATGGGCCAGCA-3′ and the other is specific for mutant allele: Rm; 5′-GCCAAAGTGATGGGCCT GCT-3′). These reverse primers are different at their 3′-end denoted in bold. The mutagenic primer (Rm) was designed to have one site mismatch (T) in order to increase the specificity of the PCR for Hb New York allele. The normal allele primer set (with primer F1 and Rn) and mutant allele primer set (F1 and Rm) amplify 303 bp fragments. Another pair of primers to amplify a part of human β-actin gene (159 bp) was included to the PCR reaction as an internal control to justify the success of amplification. These

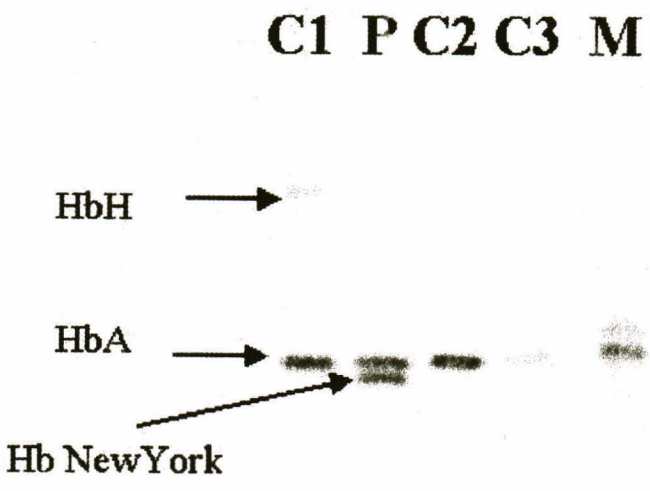

Fig. 1. Isoelectric focusing shows Hb New York running to the position right next to Hb A. (M represents Standard Hemoglobin Marker, C1 represents Hb H disease patient showing Hb H and A, P represents the proband in this report, C2 and C3 represent normal controls.)

sequences are, forward primer Fb; 5′-CGACATG GAGAAGATCTGGCAC-3′ and reverse primer Rb; 5′-GTACATGGCTG GGGTGTTGAAG-3′. The PCR reactions (normal and mutant set) were performed separately consisted of 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl (pH 8.3), 200 μM of each dNTP, 25 pmol of primer F1 and Rn (or Rm), 5 pmol of primer Fb and Rb and 2.5 U of Taq polymerase (Roche Diagnostic, Mannheim, Germany) and 100 ng of genomic DNA in a final volume of 50 μl. The reaction was carried out in MJ DNA engine thermocycler (MJ research) with an initial denaturation step of 95°C for 5 minutes and then 35 cycles of 95°C 30 seconds, 64°C 30 seconds and 72°C 90 seconds and a final extension at 72°C for 10 minutes. The PCR products were run in 2.5 per cent ethidium bromide stained agarose gel and directly visualized under an UV light transilluminator.

RESULTS

The molecular analysis of the structure of the β-globin gene using direct genomic sequencing showed a mutation of the second nucleotide of codon 113, GTG→GAG with normal sequence of codon 113 (data not shown). This result demonstrated that abnormal hemoglobin presenting in the propositus was genuine Hb New York (beta 113 (G15) Val→ Glu). Therefore, this woman is a heterozygote of Hb New York mutation. The presence of this mutation was assured using the newly developed ARMS-PCR. In the normal PCR panel (using the normal reverse primer (Rn)), a 303 bp fragment representing the amplification from normal allele was presented in the propositus (S) and two normal controls (C1 and C2) (Fig. 2). However, in the mutant PCR panel with the mutant reverse primer (Rm), a 303 bp fragment in which is specific to T→A mutation in Hb New York has been amplified successfully only in the propositus not in the controls. In both PCR panels, 159 bp-PCR fragments of β-actin gene were successfully amplified. This result confirms the heterozygosity of the proband since it has been revealed that she had positive fragments using either normal or mutant primer set. This technique can be used effectively for a rapid diagnosis for this mutation in future suspected cases.

DISCUSSION

Hb New York also known as Hb Kaohsiung was described initially in a Chinese family in New York[1,7]. Afterwards, this hemoglobin variant was repeatedly described in many Chinese descendants [8-15] and once in a Thai family[16]. To the authors' knowledge, this is the second report of such a mutation in Thailand.

The Val→Glu substitution described in Hb New York has an unremarkable effect upon the

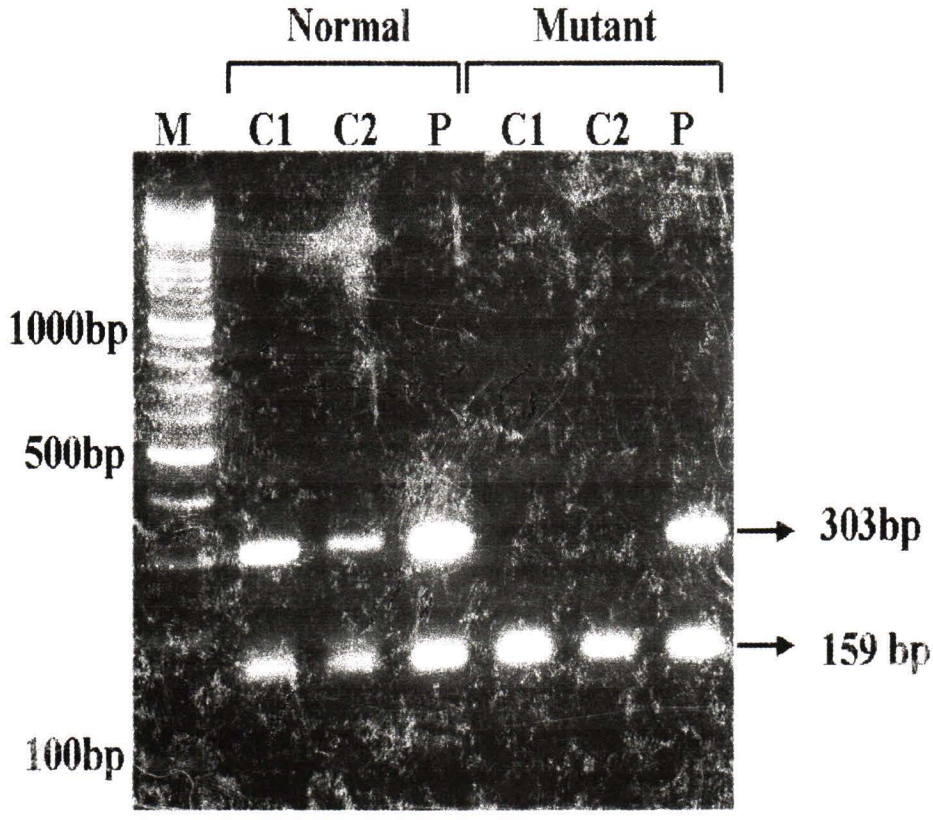

Fig. 2. A rapid and novel molecular diagnosis of Hb New York using Allele Related Mutation Specific Polymerase Chain Reaction (ARMS-PCR). (C1 and C2 represent normal controls, P represents the propositus, M = 100 Kb molecular weight marker, see text).

chemical and physical properties even though this mutation lies in the $\alpha_1\beta_1$ contact. This contact plays an important role in the maintenance of the quaternary structure of the hemoglobin molecule[11]. However, the structural alteration between oxy-Hb and deoxy-Hb by this substitution is subtle making this variant hardly distinctive from Hb A. The reversal of hydrophobic residue (Valine) to hydrophilic residue (Glutamine) results in instability of this hemoglobin molecule[8,9]. It has been shown before that the newly synthesized α-globin chain preferentially binds to this β-globin variant than to normal β-globin[8]. This leads to an increased turnover by rapid destruction of the $\alpha_2\beta_2$CD113 GTG→GAG due to the unstable property of this β-globin variant. As a result, the globin chain synthesis study was revealed showing a decrease in ratio between α/β globin syntheses[8]. Subsequently, it was demonstrated that the coinheritance of Hb New York, somewhat, augmented the clinical severity in a patient with Hb H disease by three α-globin genes deletion (--/-α) and the more discernible α/β globin chain imbalance was pronounced. This suggests that the presence of this β-globin variant may be important in modification of the clinical presentation in patients with α-thalassemia. However, a more informative study regarding clinical phenotypes of patients with double heterozygosity of Hb New York and α-thalassemia is essentially required before any conclusion can be drawn.

One drawback in the detection and diagnosis of Hb New York is this variant can hardly be discriminated from Hb A. However, the molecular characterization by direct genomic sequencing in-

cluding a novel ARMS-PCR described here a making definitive diagnosis of Hb New York feasible. It is noteworthy that this report also confirms the benefit of the isoelectric focusing technique in identifying abnormal hemoglobin in which the standard hemoglobin analyses such as starch gel electrophoresis and liquid chromatography can not detect it.

ACKNOWLEDGEMENTS

The authors wish to thank Kevin Clark and Chris Fisher for their technical assistance and Darika Seeleom for her secretarial work. V. Viprakasit was supported by a scholarship from Faculty of Medicine, Siriraj Hospital, Mahidol University. This study was partially supported by the MRC.

(Received for publication on February 1, 2002)

REFERENCES

1. Huisman THJ, Carver MFH, Efremov GD. A Syllabus of Human Hemoglobin Variant. 2nd ed. Augusta GA: The Sickle Cell Anemia Foundation, 1998.
2. Fucharoen S, Winichagoon P. Hemoglobinopathies in Southeast Asia: Molecular biology and clinical medicine. Hemoglobin 1997; 21: 299-319.
3. Marengo-Row AJ. Rapid electrophoresis and quantitation of hemoglobin on cellulose acetate. J Clin Pathol 1965; 18: 790-2.
4. Smithies O. Zone electrophoresis in starch gel: Group variations in the serum proteins of normal human adults. Biochem J 1955; 61: 629-41.
5. Fabry ME. Laboratory diagnosis of Hemoglobin disorders and animal models for their study. In Steinberg MH, Forget BG, Higgs DR, Nagel RL, eds. Disorder of Haemoglobin, 1st ed. Cambridge: Cambridge University Press, 2001: 910-40.
6. Miller SA, Dykes DD, Polesky HFA. Simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16: 1215.
7. Ranney HM, Jacobs AS, Nagel RL. Haemoglobin New York. Nature 1967; 213: 876-8.
8. Todd D, Chan V, Schneider RG, Dozy AM, Kan YW, Chan TK. Globin chain synthesis in haemoglobin New York (beta 113 replaced by glutamic acid). Br J Haematol 1980; 46: 557-64.
9. Kendall A, Pang W. Hemoglobin New York associated with alpha-thalassemia. Hum Hered 1980; 30: 50-3.
10. Zeng YT, Huang SZ. Hemoglobin New York (alpha 2 beta 2 113 (G15) Val leads to Glu) in China. Hemoglobin 1982; 6: 61-7.
11. Harano T, Harano K, Ueda S, et al. Hemoglobin New York (beta 113 (G 15) Val replaced by Glu) found in a Japanese female with cholelithiasis: Its structure, function, instability and biosynthesis. Hemoglobin 1982; 6: 619-24.
12. Chan V, Chan TK, Tso SC, Todd D. Combination of three alpha-globin gene loci deletions and hemoglobin New York results in a severe hemoglobin H syndrome. Am J Hematol 1987; 24: 301-6.
13. Yang T, Olsen KW. Thermal stability and cross-linking of Hb New York (beta 113 (G15) Val→ Glu). Hemoglobin 1989; 13: 147-56.
14. Chang JG, Lee LS, Chen PH, Chen YH. Hb Kaohsiung or New York: a T→A substitution at codon 113 of the beta-globin chain creates an Alu I cutting site. Hemoglobin 1992; 16: 123-5.
15. Ribeiro ML, Tamagnini GP. Hemoglobin disorders in Macao. Hemoglobin 1997; 21: 271-9.
16. Pootrakul S, Wasi S, Na-Nakorn S, Dixon GH. Double heterozygosity for hemoglobin E and hemoglobin New York in a Thai family. J Med Assoc Thai 1971; 54: 688-97.

การตรวจวิเคราะห์ฮีโมโกลบินนิวยอร์ค ($\beta^{CD113\ GTG\rightarrow GAG}$) ได้อย่างรวดเร็วและแม่นยำด้วยวิธี Allele Related Mutation Specific–Polymerase Chain Reaction

วิปร วิประกษิต, พ.บ.,**, กัลยา เตชะวณิชย์, วท.ม.**, เลอลักษณ์ สุวรรณฑล, วท.บ.**, ปาริฉัตร พึ่งอัมฤทธิ์, วท.บ.**, วรวุฒิ จีนช้าง, ว.ทบ.**, วรวรรณ ตันไพจิตร, พ.บ., วท.ม.***

ฮีโมโกลบินนิวยอร์ค เป็นฮีโมโกลบินที่มีความผิดปกติที่สายเบต้าโกลบิน (β-globin chain) คือ กรดอะมิโนตัวที่ 113, valine เปลี่ยนเป็น glutamic acid (GTG→GAG) ตรวจพบและรายงานครั้งแรกในครอบครัวคนจีนที่อาศัยอยู่ในนครนิวยอร์ค และยังมีรายงานภายหลังต่อมาว่า พบฮีโมโกลบินชนิดนี้ในประชากรจีนหลาย ๆ กลุ่มด้วยกัน โดยคนจีนจะเรียกว่า Hb Kaohsiung การแทนที่ของกรดอมิโนตัวที่ 113 จาก valine ไปเป็น glutamic acid นี้ มีผลเปลี่ยนแปลงต่อการเคลื่อนที่ของฮีโมโกลบินชนิดนี้ในกระแสไฟฟ้าน้อย จนทำให้การตรวจโดยวิธีมาตรฐานธรรมดา คือ วิธีแยกด้วยกระแสไฟฟ้าและวิธีโมรมาโตกราฟฟี ไม่สามารถแยกฮีโมโกลบินนิวยอร์คจากฮีโมโกลบินเอได้ ในรายงานนี้ได้พัฒนาวิธีตรวจหาความผิดปกติของฮีโมโกลบินนิวยอร์ค โดยใช้วิธี Allele Related Mutation Specific–Polymerase Chain Reaction (ARMS–PCR) ซึ่งเป็นเทคนิคที่สามารถทำได้อย่างรวดเร็วและจำเพาะ

คำสำคัญ : ฮีโมโกลบินนิวยอร์ค, ความผิดปกติของเบต้าโกลบิน, ฮีโมโกลบินผิดปกติ

วิปร วิประกษิต, กัลยา เตชะวณิชย์, เลอลักษณ์ สุวรรณฑล, ปาริฉัตร พึ่งอัมฤทธิ์, วรวุฒิ จีนช้าง, วรวรรณ ตันไพจิตร
จดหมายเหตุทางแพทย์ ฯ 2545; 85 (ฉบับพิเศษ 2): S558–S563

* MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, UK.

** หน่วยโลหิตวิทยา, ภาควิชากุมารเวชศาสตร์, คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล, กรุงเทพ ฯ 10700