REFERENCES

1. Weatherall DJ, Clegg JB. **The thalassemia syndromes**. 4 ed. Oxford, UK: Blackwell Science; 2001.
2. Changtrakun Y, Fucharoen S, Ayukarn K, Siriratmanawong N, Fucharoen G, Sanchaisuriya K. Compound heterozygosity for Hb Korle-Bu (β^{73}; Asp-Asn) and Hb E (β^{26}; Glu-Lys) with a 3.7 kb deletional α-thalassemia in Thai patients. **Ann Hematol** 2002; 81: 389-93.
3. Higgs DR. alpha-Thalassemia. **Baillieres Clin Haematol** 1993; 6(1): 117-50.
4. Wasi P, Pootrakul P, Pravatmung P, Winichagoon P, Fucharoen S. Thalassemia in Thailand. **Ann N Y Acad Sci** 1980; 344: 352-63.
5. Bunn HF, Forget BG, Ranney HM. **Human hemoglobins**. USA: W.B. Saunders; 1997.
6. Gallerani M, Cicognani I, Ballardini P, Savelli S, Martinelli L, Ricci A, et al. Average life expectancy of heterozygous β-thalassemic subjects. **Haematologica** 1990; 75: 224-7.
7. Fucharoen S, Winichagoon P. Hemoglobinopathies in Southeast Asia: molecular biology and clinical medicine. **Hemoglobin** 1997; 21: 299-319.
8. Fucharoen S, Winichagoon P. Hemoglobinopathies in Southeast Asia. **Hemoglobin** 1987; 11: 65-88.
9. Bianchi DW. PCR quantitation of fetal cells in maternal blood in normal and aneuploid pregnancies. **Am J Hum Genet** 1997; 61: 822-9.
10. Lo YM, Tein M, Lau T, Haines C, Leung T, Poon P, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. **Am J Hum Genet** 1998; 62: 768-75.
11. Costa JM, Cenachi A, Gautier E. New strategy for prenatal diagnosis of X-linked disorders. **N Engl J Med** 2002; 346: 1734-8.
12. Lo YM, Hjelm NM, Fidler C, Sargent IL, Murphy MF, Chamberlain PF, et al. Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. **N Engl J Med** 1998; 339: 1734-8.

13. Saito H, Sekizawa A, Morimoto T, Suzuki M, Yanaihira T. Prenatal DNA diagnosis of a sigle-gene disorder from maternal plasma. **Lancet** 2000; 356: 1170.

14. Chen CP, Chern SR, Wang W. Fetal DNA in maternal plasma: the prenatal detection of a paternally inherited fetal aneuploidy. **Prenat Diagn** 2000; 20: 355-7.

15. Leung TN, Zhang J, Lau TK, Chan AY, Lo YM. Increased maternal plasma fetal DNA concentrations in women who eventually develop preeclamsia. **Clin Chem** 2001; 47: 137-9.

16. Swinkels DW. Hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome as a complication of preeclampsia in pregnant women increases the amount of cell-free fetal and maternal DNA in maternal plasma and serum. **Clin Chem** 2002; 48: 650-3.

17. Svasti J, Srisomsap C, Winichagoon P, Fucharoen S. Detection and structural analysis of abnormal hemoglobins found in Thailand. **Southeast Asian J Trop Med Public Health** 1999; 30 Suppl 2: 88-93.

18. Liang ST, Wong VC, So WW, Ma HK, Chan V, Todd D, et al. Homozygous alpha-thalassaemia: clinical presentation, diagnosis and management. A review of 46 cases. **Br J Obstet Gynaecol** 1985; 92: 680-4.

19. Nakayama R, Yamada D, Steinmiller V, Hsia E, Hale RW. Hydrops fetalis secondary to Bart hemoglobinopathy. **Obstet Gynecol** 1986; 67: 176-80.

20. Vaeusorn O, Fucharoen S, Ruangpiroj T, al. e. Fetal pathology and maternal morbidity in hemoglobin Bart's hydrops fetalis: an analysis of 65 cases. In: First International Conference on Thalassemia, Bangkok. 1985.

21. Guy G, Coady DJ, Jansen V, Snyder J, Zinberg S. α-Thalassemia hydrops fetalis: clinical and ultrasonographic considerations. **Am J Obstet Gynecol** 1985; 153: 500-4.

22. Brumfield CG, Atkinson MW. Invasive techniques for fetal evaluation and treatment. **Clin Obstet Gynecol** 1994; 37: 856-74.

23. Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, et al. Presence of fetal DNA in maternal plasma and serum. **Lancet** 1997; 350: 485-7.

24. Honda H, Miharu N, Ohashi Y, Samura O, Kinutani M, Hara T, et al. Fetal gender determination in early pregnancy through qualitative and quantitative analysis of fetal DNA in maternal serum. **Hum Genet** 2002; 110: 75-9.

25. Pertl B, Sekizawa A, Samura O, Orescovic I, Rahaim PT, Bianchi DW. Detection of male and female fetal DNA in maternal plasma by multiplex fluorescent polymerase chain reaction amplification of short tandem repeats. **Hum Genet** 2000; 106: 45-9.

26. Amicucci P, Gennarelli M, Novelli G, Dallapiccola B. Prenatal diagnosis of myotonic dystrophy using fetal DNA obtained from maternal plasma. **Clin Chem** 2000; 46: 301-2.

27. Gonzalez-Gonzalez MC, Garcia-Hoyos M, Trujillo MJ, Rodriguez de Alba M, Lorda-Sanchez I, Diaz-Recasens J, et al. Prenatal detection of a cystic fibrosis mutation in fetal DNA from maternal plasma. **Prenat Diagn** 2002; 22: 946-8.

28. Chiu RW, Lau TK, Cheung PT, Gong ZQ, Leung TN, Lo YM. Noninvasive prenatal exclusion of congenital adrenal hyperplasia by maternal plasma analysis: a feasibility study. **Clin Chem** 2002; 48: 778-80.

29. Chiu RW, Lau TK, Leung TN, Chow KC, Chui DH, Lo YM. Prenatal exclusion of beta thalassaemia major by examination of maternal plasma. **Lancet** 2002; 360: 998-1000.

30. Tungwiwat W, Fucharoen S, Fucharoen G, Ratanasiri T, Sanchaisuriya K. Development and application of a real-time quantitative PCR for prenatal detection of fetal alpha(0)-thalassemia from maternal plasma. **Ann N Y Acad Sci** 2006; 1075: 103-7.

31. Lo YM, Leung TN, Tein MS, Sargent IL, Zhang J, Lau TK, et al. Quantitative abnormalities of fetal DNA in maternal serum in preeclampsia. **Clin Chem** 1999; 45: 184-8.

32. Zhong XY, Laivuori H, Livingston JC, Ylikorkala O, Sibai BM, Holzgreve W, et al. Elevation of both maternal and fetal extracellular circulating deoxyribonucleic acid concentrations in the plasma of pregnant women with preeclamsia. **Am J Obstet Gynecol** 2001; 184: 414-9.

33. Higgs DR, Vickers MA, Wilkie AO, Pretorius IM, Jarman AP, Weatherall DJ. A review of the molecular genetics of the human alpha-globin gene cluster. **Blood** 1989; 73: 1081-4.

34. Beaudry MA, Ferguson DJ, Pearse K, Yanofsky RA, Rubin EM, Kan YW. Survival of a hydropic infant with homozygous alpha-thalassemia-1. **J Pediatr** 1986; 108: 713-6.

35. Lau YL, Chan LC, Chan YY, Ha SY, Yeung CY, Waye JS, et al. Prevalence and genotypes of α- and β-thalassemia carriers in Hong Kong :implications for population screening. **N Engl J Med** 1997; 336: 1298-301.

36. Hsieh FJ, Ko TM, Chen HY. Hydrops fetalis caused by severe alpha-thalassemia. **Early Hum Dev** 1992; 29: 233-6.

37. Modell B, ed. **Guidelines for the Control of Haemoglobin Disorders**. Geneva: World Health Organization Hereditary Diseases Programme; 1994.

38. Kitsirisakul B, Steger HF, Sanguansermsri T. Frequency of alpha-thalassemia-1 of the Southeast Asian-type among pregnant women in northern Thailand determined by PCR technique. **Southeast Asian J Trop Med Public Health** 1996; 27: 362-3.

39. Taylor JM, Dozy A, Kan YW, Varmus HE, Lie-Injo LE, Ganesan J, et al. Genetic lesion in homozygous alpha thalassaemia (hydrops fetalis). **Nature** 1974; 251: 392-3.

40. Ottolenghi S, Lanyon WG, Paul J, Williamson R, Weatherall DJ, Clegg JB, et al. The severe form of alpha thalassaemia is caused by a haemoglobin gene deletion. **Nature** 1974; 251: 389-92.

41. Fischel-Ghodsian N, Vickers MA, Seip M, Winichagoon P, Higgs DR. Characterization of two deletions that remove the entire human zeta-alpha globin gene complex (- -THAI and - -FIL). **Br J Haematol** 1988; 70: 233-8.

42. Peschle C, Mavilio F, Carè A, Migliaccio G, Migliaccio AR, Salvo G, et al. Haemoglobin switching in human embryos: asynchrony of zeta-alpha and epsilon-gamma-globin switches in primitive and definite erythropoietic lineage. **Nature** 1985; 313: 235-8.

43. Kazazian HJ, Woodhead AP. Hemoglobin A synthesis in the developing fetus. **N Engl J Med** 1973; 289: 58-62.

44. Weatherall DJ. Thalassemias. In: Beutler E, Lichtman MA, Coller BS, Kipps TJ, editors. **Williams Hematology**. 5 ed. New York, NY, McGraw-Hill; 1997.

45. Chui DH, Waye JS. Hydrops fetalis caused by alpha-thalassemia: an emerging health care problem. **Blood** 1998; 91: 2213-22.

46. Olivieri NF. Fetal erythropoiesis and the diagnosis and treatment of hemoglobin disorders in the fetus and child. **Semin Perinatol** 1997; 21: 63-9.

47. Bunn HF. Human hemoglobins: Normal and abnormal; methemoglobinemia. In: Nathan DG, Oski FA, editors. **Hematology of Infancy and Childhood**. 4 ed. Canada: W.B. Saunders; 1993. p. 711.

48. Ghosh A, Tang MHY, Lam YH, Fung E, Chan V. Ultrasound measurement of placental thickness to detect pregnancies affected by α-thalassaemia-1. **Lancet** 1994; 344: 988-9.

49. Mehr DS, Rector JT, Ngo K-Y. Pathological case of the month. Hydrops fetalis secondary to homozygous α-thalassemia-1 (Bart's hemoglobinopathy). **Arch Pediatr Adolesc Med** 1994; 148: 1313-4.

50. Hahn S, Gupta AK, Troeger C, Rusterholz C, Holzgreve W. Disturbances in placental immunology: ready for therapeutic interventions?. **Semin Immunopathol** 2006; 27: 477-93.

51. Huppertz B. The feto-maternal interface: setting the stage for potential immune interactions. **Semin Immunopathol** 2007; 29: 83-94.

52. Jauniaux E, Poston L, Burton GJ. Placental-related diseases of pregnancy: Involvement of oxidative stress and implications in human evolution. **Hum Reprod Update** 2006; 12: 747-55.

53. Hung TH, Skepper JN, Charnock-Jones DS, Burton GJ. Hypoxia-reoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia. **Circ Res** 2002; 90: 1274-81.

54. Soleymanlou N, Jurisica I, Nevo O, Ietta F, Zhang X, Zamudio S, et al. Molecular evidence of placental hypoxia in preeclampsia. **J Clin Endocrinol Metab** 2005; 90: 4299-308.

55. Maynard SE, Min JY, Merchan J, Lim KH. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. **J Clin Invest** 2003; 111: 649-58.

56. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. **Science** 2005; 308: 1592-4.

57. Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, McLaughlin MK. Preeclampsia: an endothelial cell disorder. **Am J Obstet Gynecol** 1989; 161: 1200-4.

58. Roberts JM, Hubel CA. The two stage model of preeclampsia: variations on the theme. **Placenta** 2009; 30(Suppl A): 32-7.

59. Levine RJ, Qian C, Leshane ES, K.F. Y, England LJ, Schisterman EF, et al. Two-stage elevation of cell-free fetal DNA in maternal sera before onset of preeclamsia. **Am J Obstet Gynecol** 2004; 190: 707-13.

60. Lau ET, Kwok YK, Luo HY, Leung KY, Lee CP, Lam YH, et al. Simple non-invasive prenatal detection of Hb Bart's disease by analysis of fetal erythrocytes in maternal blood. **Prenat Diagn** 2005; 25(2): 123-8.

61. Fucharoen G, Tungwiwat W, Ratanasiri T, Sanchaisuriya K, Fucharoen S. Prenatal detection of fetal hemoglobin E gene from maternal plasma. **Prenat Diagn** 2003; 23: 393-6.

62. Tungwiwat W, Fucharoen G, Fucharoen S, Ratanasiri T, Sanchaisuriya K, Sae-Ung N. Application of maternal plasma DNA analysis for noninvasive prenatal diagnosis of Hb E-beta-thalassemia. **Transl Res** 2007; 150(5): 319-25.

63. Bianchi DW. Fetal cells in the maternal circulation feasibility for prenatal diagnosis. **Br J Haematol** 1999; 105: 574-83.

64. Bianchi DW. Fetal DNA in maternal plasma: the plot thickens and the placental barrier thins. **Am J Hum Genet** 1998; 62: 763-4.

65. Thomas MR, Williamson R, Craft I, Yazdani N, Rodeck CH. Y chromosome sequence DNA amplified from peripheral blood of woman in early pregnancy. **Lancet** 1994; 343: 413-4.

66. Thomas MR, Tutschek B, Frost A, Rodeck CH, Yazdani N, Craft I, et al. The time of appearance and disappearance of fetal DNA from the maternal circulation. **Prenat Diagn** 1995; 15: 641-6.

67. Bianchi DW, Stewart JE, Garber MF, Lucotte G, Flint AF. Possible effect of gestational age on the detection of fetal nucleated erythrocytes in maternal blood. **Prenat Diagn** 1991; 11: 523-8.

68. Sekizawa A, Samura O, Zhen DK, Falco V, Farina A, Bianchi DW. Apoptosis in fetal nucleated erythrocytes circulating in maternal blood. **Prenat Diagn** 2000; 20: 886-69.

69. Lo YM, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. **Am J Hum Genet** 1999; 64: 218-24.

70. Hamada H, Arinami T, Kubo T, Hamaguchi H, Iwasaki H. Fetal nucleated cells in maternal peripheral blood: frequency and relationship to gestational age. **Hum Genet** 1993; 91: 427-32.

71. Moore and Persaud. The Developing Human Clinically Orientated Embryology. 6 ed.; 1997.

72. Zhong XY, Burk MR, Troeger C, Kang A, Holzgreve W, Hahn S. Fluctuation of maternal and fetal free extracellular circulatory DNA in maternal plasma. **Obstet Gynecol** 2000; 96: 991-6.

73. Lo YM, Tein M, Lau T, Haines C, Leung T, Poon P, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. **Am J Hum Genet** 1998; 62: 768-75.

74. Hahn S, Holzgreve W, editors. **Fetal Cells and Fetal DNA in Maternal Blood New Developments for a New Millinium**. Proceedings of the 11th Fetal Cell Workshop; 2000 Apr 15; Basel, Switzerland.

75. Sekizawa A, Jimbo M, Saito H. Cell-free fetal DNA in the plasma of pregnant women with severe fetal growth restriction. **Am J Obstet Gynecol** 2003; 188: 480-4.

76. Sirover MA. New insights into an old protein: the functional diversity of mammalian glyceraldehyde-3-phosphate dehydrogenase. **Biochim Biophys Acta** 1999; 1432: 159-84.

77. Glaser PE, Han X, Gross RW. Tubulin is the endogenous inhibitor of the glyceraldehyde 3-phosphate dehydrogenase isoform that catalyzes membrane fusion: Implications for the coordinated regulation of glycolysis and membrane fusion. **Proc Natl Acad Sci USA** 2002; 99: 14104-9.

78. Berry MD, Boulton AA. Glyceraldehyde-3-phosphate dehydrogenase and apoptosis. **J Neurosci Res** 2000; 60: 150-4.

79. Sundararaj KP, Wood RE, Ponnusamy S, Salas AM, Szulc Z, Bielawska A, et al. Rapid shortening of telomere length in response to ceramide involves the inhibition of telomere binding activity of nuclear glyceraldehyde-3-phosphate dehydrogenase. **J Biol Chem** 2004; 279: 6152-62.

80. Dharmaraj S. **Real-Time PCR chemistries**. [online] 2009 [cited 2009 June 22]. Available form: http://www.ambion.com/ techlib/basics/rtpcr/index.html.

81. Clegg JB, Weatherall DJ, Gibbons R, Higgs DR, Old JM, et al. Human hemoglobin. **The thalassemia syndromes**. 4 ed. Oxford: Blackwell Science; 2001. p. 65-120.

82. Fucharoen S, Fucharoen G, Sae-ung N, Sanchaisuriya K, Fukumaki Y. Molecular and hematological characterization of Hb Tak and Hb Pyrgos in Thailand. **Southeast Asian J Trop Med Public Health** 1997; 28(Suppl.3): 110-4.

83. Hoyer JD, Wick MJ, Thibodeau SN, Viker KA, Conner R, Fairbanks VF. Hb Tak confirmed by DNA analysis: not expressed as thalassemia in a Hb Tak / Hb E compound heterozygote. **Hemoglobin** 1998; 22(1): 45-52.

84. Boontrakoonpoontawee P, Svasti J, Fucharoen S, Winichagoon P. Identification of Hb Lepore-Washington-Boston in association with Hb E [beta26(B8)Glu-->Lys] in a Thai female. **Hemoglobin** 1987; 11(4): 309-16.

85. Viprakasit V, Pung-Amritt P, Suwanthon L, Clark K, Tanphaichitr VS. Complex interactions of δβ hybrid haemoglobin (Hb Lepore-Hollandia) Hb E($\beta^{26G-->A}$) and α^{+} thalassemia in a Thai family. **Eur J Haematol** 2002; 68: 107-11.

86. Fucharoen S, Winichagoon P. Thalassemia and abnormal hemoglobin. **Int J Hematol** 2002; 76 (suppl2): 83-9.

87. Hardison RC, Chui DHK, Giardine B, Riemer C, Patrinos GP, Anagnou N, et al. Hb Var. a relational database of human hemoglobin variants and thalassemia mutations at the globin gene server. **Hum Mutat** 2002; 19: 225-33.

88. Zeng FY, Fucharoen S, Huang SZ, Rodgers GP. Hb Q-Thailand [alpha 74(EF3) Asp→His]: gene organization, molecular structure, and DNA diagnosis. **Hemoglobin** 1992; 16(6): 481-91.

89. Beris P, Huber P, Miescher PA, Wilson JB, Kutlar A, Chen SS, et al. Hb Q-Thailand - Hb H disease in a Chinese living in Geneva, Switzerland: characterization of the variant and identification of the two alpha-thalassemic chromosomes. **Am J Hematol** 1987; 24(4): 395-400.

90. Higgs DR, Hunt HC, Drysdale HC, J.B. C, Pressley L, Weatherall DJ. The genetic basis of Hb Q-H disease. **Br J Haematol** 1980;46:387-400.

91. Leung KF, Ma ES, Chan AY, Chan LC. Clinical phenotype of haemoglobin Q-H disease. **J Clin Pathol** 2004; 57(1): 81-2.

92. Sanchaisuriya K, Chunpanich S, Fucharoen S, Fucharoen G, Sanchaisuriya P, Changtrakun Y. Association of Hb Q-Thailand with homozygous Hb E and heterozygous Hb Constant Spring in pregnancy. **Eur J Haematol** 2005; 74(3): 221-7.

93. Pootrakul S, Srichayanont S, Wasi P, Suanpan S. Hemoglobin Siam ($\alpha_2^{15Arg}\beta_2$): a new α-chain variant. **Hum Genet** 1974; 23: 199-204.

94. Yodsowan B, Svasti J, Srisomsap C, Winichagoon P, Fucharoen S. Hb Siam [α15(A13)Gly→Asp] is a GGT→CGT mutation in the α1-globin gene. **Hemoglobin** 2000; 24(1): 71-5.

95. Tatsis B. Hemoglobin Queens (α34 (B15)Leu-Arg): A new variant at the α1β1 contact. **Blood** 1979; 54 (Suppl1): 61a.

96. Fucharoen S, Singsanan S, Hama A, Fucharoen G, Sanchaisuriya K. Rapid molecular characterization of Hb Queens and Hb Siam: Two variants easily misidentified as sickle Hb. **Clin Biochem** 2007; 40: 137-40.

97. Moo-Penn WF, Jue DL, Johnson MH, McGuffey JE, Simpkins H, Katz J. Hemoglobin Queens: a34 (B15) leu-arg. Structural and functional properties and its association with Hb E. **Am J Hematol** 1982; 13: 323-7.

98. Sugihara J, Imamura T, Yamada H, Imoto T, Matsuo T, Sumida I, et al. A new electropholetic variant of Hemoglobin (Ogi) in which a leucine residue is replaced by an arginine residue at position 34 of the α-chain. **Biochim Biophys Acta** 1982; 701: 45-8.

99. Yongsuwan S, Svasti J, Fucharoen S. Decreased heat stability found in purified hemoglobin Queens (alpha34 (B15) Leu-Arg). **Hemoglobin** 1987; 11: 567-70.

100. Huisman TH, Carver MFH, Efremov GD. **A syllabus of human hemoglobin variants.** USA: The Sickle Cell Anemia Foundation; 1996.

101. Harano T, Harano K, Imai N, Ueda S, Seki M. An electrophoretically silent hemoglobin variant, Hb Hekinan [α27(B8)Glu-Asp] found in Japanese. **Hemoglobin** 1988; 12: 61-5.

102. Zhao W, Wilson JB, Webber BB, Kutlar A, Tamagnini GP, Kuam B, et al. Hb Hekinan observed in three Chinese from Macau; identification of the GAG-GAT mutation in the α1-globin gene. **Hemoglobin** 1990; 14: 627-35.

103. Merault G, Keclard L, Desfontaines L, Saint-Martin C, Blouquit Y, Rosa J, et al. Hemoglobin Hekinan [alpha(2)27(B8)Glu-Asp beta2] detected in Guyana. **Hemoglobin** 1989; 13: 397-402.

104. Fucharoen S, Changtrakun Y, Ratanasiri T, Fucharoen G, Sanchaisuriya K. Complex interaction of Hb Hekinan [α27(B8)Glu-Asp] and Hb E[β26(B8) Glu-Lys] with a deletional α-thalassemia 1 in a Thai family. **Eur J Haematol** 2003; 70: 304-9.

105. Liang CC, Chen S, Yang K, Jia P, Ma Y, Li T, et al. Hemoglobin Beijing [a16(A14)Asn-->Lys]: a new fast-moving hemoglobin variant. **Hemoglobin** 1982; 6(6): 629-33.

106. Fucharoen S, Chunpanich S, Sanchaisuriya K, Fucharoen G, Kunyanone N. Thalassemia intermedia associated with complex interaction of Hb Beijing [alpha16(A14)Lys-->Asn] and Hb E [beta26(B8)Glu-->Lys] with a deletional alpha-thalassemia-1 in a Thai family. **Hemoglobin** 2005; 29(1): 77-83.

107. Bunn HF, Forget B. **Hemoglobin: Molecular, Genetic and Clinical Aspects**. USA: W.B. Saunders; 1986.

108. Boehm CD, Dowling CE, Antonarakis SA, Honig GR, Kazazian HH. Evidence supporting a single origin of the β^C-globin gene in blacks. **Am J Hum Genet** 1985; 37: 771-7.

109. Weatherall DJ, Clegg JB. **The thalassemia syndromes**. 3 ed. Oxford: Blackwell Scientific Publications; 1981.

110. Bachir D, Galacteros F. Hemoglobin C. **Orplanet Encyclopedia** 2004:1-4.

111. Flatz G, Kinderlerer JL, Kilmartin JV, Lehmann H. Haemoglobin Tak: a variant with additional residues at the end of the β-chains. **Lancet** 1971; 1: 732-3.

112. Imai K, Lehmann H. The oxygen affinity of haemoglobin Tak, a variant with an elongated β-chain. **Biochim Biophys Acta** 1975; 412: 288-94.

113. Imai K, Tientadakul P, Opartkiattikul N, Luenee P, Winichagoon P, Svasti J, et al. Detection of haemoglobin variants and inference of their functional properties using complete oxygen dissociation curve measurements. **Br J Haematol** 2001; 112: 483-7.

114. Brittenham GM. Globin gene variants and polymorphisms in India. In: Winter DJ, editor. **Hemoglobin Variants in Human Populations**. USA: CRC Press; 1987. p. 79-110.

115. Li H, Zhao X, Qin F, Li HW, Li L, He X, et al. Abnormal hemoglobins in the Silk Road Region of China. **Hum Genet** 1990; 86: 231-5.

116. El-Kella S, Mathews AR. Hb D-Punjab in the United Arab Emirates. **Hemoglobin** 1997; 21(4): 369-75.

117. Fucharoen S, Changtrakun Y, Surapot S, Fucharoen G, Sanchaisuriya K. Molecular characterization of Hb D-Punjab [β121(GH4)Glu-Gln] in THailand. **Hemoglobin** 2002; 26(3): 261-9.

118. Mukherjee MB, Surve RR, Gangakhedkar RR, Mohanty D, Colah RB. Hemoglobin sickle D Punjab. **Ind J Hum Genet** 2005; 11 (3): 154-5.

119. Konotey-Ahulu FID, Gallo E, Lehmann H, Ringelhann B. Haemoglobin Korle-Bu (β73 aspatic acid-asparagine) showing one of the two amino acid substitutions of haemoglobin C Harlem. **J Med Genet** 1968; 5: 107-11.

120. Fabritius H, Millan J, Corroller Y. Systematic screening of hemoglobinopathies in blood donors in Guadeloupe. **Rev Fr Transfus Immunohematol** 1978; 21: 937-50.

121. Honig GR, Seeler RA, Shamsuddin M, Vida LN, Mompoint M, Valcourt E. Hemoglobin Korle-Bu in Mexican family. **Hemoglobin** 1983; 7: 185-9.

122. Boissel JP, Fabritius H, Richard P, Wajcman H, Cabannes R, Labie D. Polymorphism of hemoglobin D in Ivory Coast: Hb Korle-Bu (beta 73(E17)Asp leads to Asn), Hb Avicenna (beta47 (CD6) Asp leads to Ala) and Hb Cocody. **Nouv Rev Fr Hematol** 1981; 23: 197-201.

123. Milner PE. High incidence of haemoglobin G Accra in a rural district in Jamaica. **J Med Genet** 1967; 4: 88-90.

124. Nagel RL, Lin MJ, Witkowska E, Fabry ME, Bestak M, Hirsch E. Compound heterozygosity for hemoglobin C and Korle-Bu: moderate microcytic anemia and acceleration of crystal formation. **Blood** 1993; 82: 1907-12.

125. Minnichi V, Hill RJ, Khuri PD, Anderson ME. Hemoglobin Hope: A Beta Chain Variant. **Blood** 1965; 25: 830-8.

126. Ingle J, Adewoye A, Dewan R, Okoli M, Rollins L, Eung SH, et al. Hb Hope [beta136(H14)Gly-->Asp (GGT-->GAT)]: interactions with Hb S [beta6(A3) Glu-->Val (GAG-->GTG)], other variant hemoglobins and thalassemia. **Hemoglobin** 2004; 28(4): 277-85.

127. Pagnier J, Gacon G, Wajcman H, Labie D. Association of Hb Hope with beta (0) thalassemia. **Hemoglobin** 1978; 2(5): 457-62.

128. Charache S, Achuff S, Winslow R, Kazazian H. Oxygen transport in a woman with hemoglobin Hope/beta+ thalassemia. **J Lab Clin Med** 1979; 93(2): 316-20.

129. Martinez G, Colombo B. Interaction between Hb S and Hb Hope in a Cuban family. **Hemoglobin** 1984; 8(5): 519-22.

130. Pillers DA, Jones M, Head C, Jones RT. Hb Hope [beta 136(H14) Gly----Asp] and Hb E [beta 26(B8)Glu----Lys]: compound heterozygosity in a Thai Mien family. **Hemoglobin** 1992; 16(1-2): 81-4.

131. Rahbar S, Nozari G, Asmerom Y, Martin PA, Yeh CH, Lee TD. Association of Hb Hope [beta 136(H14)Gly----Asp] and alpha-thalassemia-2 (3.7 Kb deletion) causing severe microcytic anemia. **Hemoglobin** 1992; 16(5): 421-5.

132. Svasti S, Yodsowon B, Sriphanich R, Winichagoon P, Boonkhan P, Suwanban T, et al. Association of Hb Hope [beta136(H14)Gly-->Asp] and Hb H disease. **Hemoglobin** 2001; 25(4): 429-35.

133. Deyde VM, Lo BB, Aw T, Fattoum S. HbHope/HbS and HbS/beta-thal double compound heterozygosity in a Mauritanian family: clinical and biochemical studies. **Ann Hematol** 2003; 82(7): 423-6.

134. Chunpanich S, Fucharoen S, Sanchaisuriya K, Fucharoen G, Kam-itsara K. Molecular and hematological characterization of hemoglobin Hope/hemoglobin E and hemoglobin Hope/alpha-thalassemia 2 in Thai patients. **Lab Hematol** 2004; 10(4): 215-20.

135. Beneitez D, Carrera A, Duran-Suarez JR, Paz V, Leon A, Garcia Talavera J. Heterozygous Hb Hope [beta136(H14)Gly --> Asp] in association with heterozygous beta0-thalassemia with apparent homozygous expression, in a Spanish patient. **Hemoglobin** 2006; 30(1): 45-9.

136. Tatsis B, Sofroniadou K, Stergiopoulos CI. Hemoglobin Pyrgos alpha2 beta2 83(EF7) Gly leads to Asp; a new hemoglobin variant in double heterozygosity with hemoglbin S. **Blood** 1976; 47: 827-32.

137. Yamada H, Hotta T, Ohba Y, Miyaji T, Ito J, Minami M. Hemoglobin Pyrgos (β83 Gly replaced by Asp) in a Japanese family. **Hemoglobin** 1977; 1: 245-55.

138. Wajcman H, Gacon G, Tudury C, Labie D, Le QY, D.H. T. Hemoglobin Pyrgos β83 (EF7) Gly leads to Asp in a Malian: structural identification and functional properties. **Nouv Rev Fr Hematol** 1978; 25: 403-11.

139. Schilliro G, Russo-Mancuso G, Dibenedetto SP, Shaperi P, Catalda A, Ragusa R, et al. Six rare hemoglobin variants found in Sicily. **Hemoglobin** 1991; 15: 431-7.

140. Qin WB, Yue XL, Qin LY, Ju TL, Wilson JB, Gu LH, et al. Two rare hemoglobin variants: He Pyrgos [β83(EF7)Gly-Asp] and Hb Legnano [α141 (Hc3)Arg-Leu] found in Inner Mongolia, P.R. China.
Hemoglobin 1994;18:343-5.

141. Jetsrisuparb A, Sanchaisuriya K, Fucharoen G, Fucharoen S, Wiangnon S, Komwilaisak P. Triple heterozygosity of a hemoglobin variant; hemoglobin Pyrgos with other hemoglobinopathies. **Int J Hematol** 2002; 75: 35-9.

142. Sawangareetrakul P, Svasti S, Yodsowan B, Winichagoon P, Srisomsap C, Svasti J, et al. Double heterozygosity for Hb Pyrgos [β83(EF7)Gly-Asp] and Hb E [β26(B8)Glu-Lys] found in association with α-thalassemia. **Hemoglobin** 2002; 26: 191-6.

143. Pootrakul P, Gray GR, Dixon GH. Hemoglobin J Bangkok in a Chinese Canadian newborn. **Can J Biochem** 1970; 48: 1370-6.

144. Honig GR, Shamsuddin M, Vida LN, Mompoint M, Valcourt E, Borders M. A third American black family with Hb J Bangkok: association of Hb J Bangkok with Hb C. **Hemoglobin** 1982; 6: 635-9.

145. Iuchi I, Hidaka K, Shimasaki S, Shibata S, Ueda S, Mizushima M, et al. Abnormal hemoglobins in the Takamatsu district with emphasis on epidemiological characteristics. **Hemoglobin** 1982; 6: 493-502.

146. Chang JG, Shih MC, Lui SC, Chen CM, Chan WL, Lee TP, et al. Hb G-Honolulu [α30(B11)Glu-Gln(α2)], Hb J-Meinung [β56(D7)Gly-Asp], and β-thalassemia [odons41/42(-TCTT)] in a Taiwanese family. **Hemoglobin** 2002; 26: 325-8.

147. Fucharoen S, Ayukarn K, Sanchaisuriya K, Fucharoen G. A typical hemoglobin H disease in a Thai patientttt resulting from a combination of α-thalassemia 1 and hemoglobin Constant Spring with hemoglobin J-Bangkok heterozygosity. **Eur J Haematol** 2001; 66: 312-6.

148. Louahabi A, Philippe M, Lali S, Wallemacq P, Maisin D. Evaluation of a new Sebia kit for analysis of hemoglobin fractions and variants on the Capillarys system. **Clin Chem Lab Med** 2006; 44(3): 340-5.

149. Chunpanich S. **Molecular characterization of two abnormal hemoglobins found in Thailand**: Master thesis in Medical Science, Khon Kaen University: Associated Medical Science; 2004.

150. Fucharoen G, Fucharoen S, 186-7. Rapid and simultaneous non-radioactive method for detecting alpha-thalassemia 1 (SEA type) and Hb Constant Spring genes. **Eur J Haematol** 1994; 53: 186-7.

151. Panyasai S, Sringam P, Fucharoen G, Sanchaisuriya K, Fucharoen S. A simplified screening for alpha-thalassemia 1 (SEA type) using a combination of a modified osmotic fragility test and a direct PCR on whole blood cell lysates. **Acta Haematol** 2002; 108: 74-8.

152. Fucharoen S, Fucharoen G, Sanchaisuriya K, Pengjam Y. Molecular analysis of a thai beta-thalassaemia heterozygote with normal haemoglobin A2 level: implication for population screening. **Ann Clin Biochem** 2002; 39: 44-9.

153. Fucharoen S, Fucharoen G, Fukumaki Y. Simple non-radioactive method for detecting haemoglobin Constant Spring gene. **Lancet** 1990; 335: 1527.

154. Fucharoen S, Sanchaisuriya K, Fucharoen G, Panyasai S, Devenish R, Luy L. Interaction of hemoglobin E and several forms of alpha-thalassemia in Cambodian families. **Haematologica** 2003; 88: 1092-8.

155. Sanchaisuriya K, Fucharoen G, Fucharoen S. Hb Pakse' [(alpha2) codon 142 (TAA→TAT or Term→Tyr)] in Thai patients with EABart's disease and Hb H Disease. **Hemoglobin** 2002; 26: 227-35.

156. Fucharoen S, Fucharoen G, Sriroongrueng W, Laosombat V, Jetsrisuparb A, Prasatkaew S, et al. Molecular basis of beta-thalassemia in Thailand: analysis of beta-thalassemia mutations using the polymerase chain reaction. **Hum Genet** 1989; 84: 41-6.

157. Sanchaisuriya K, Chunpanich S, Fucharoen G, Fucharoen S. Multiplex allele specific PCR assay for differential diagnosis of Hb S, Hb D-punjab and Hb Tak. **Clinica Chimica Acta** 2004; 343: 129-34.

158. Chunpanich S, Fucharoen S, Sanchaisuriya K, Fucharoen G, Kam-itsara K. Molecular and hematological characterization of hemoglobin Hope/hemoglobin E and hemoglobin Hope/alpha-thalassemia 2 in Thai patients. **Lab Hematol** 2004; 10: 215-20.

159. Fucharoen S, Singsanan S, Sanchaisuriya K, Fucharoen G. Molecular and haematological characterization of compound Hb E/Hb Pyrgos and Hb E/Hb J-Bangkok in Thai patients. **Clin Lab Haematol** 2005; 27: 184-9.

160. Fucharoen S, Chunpanich S, Sanchaisuriya K, Fucharoen G, Kunyanone N. Thalassemia intermedia associated with complex interaction of Hb Beijing [alpha16(A14)Lys→Asn] and Hb E [beta26(B8)Glu→Lys] with a deletional alpha-thalassemia-1 in a Thai family. **Hemoglobin** 2005; 29: 77-83.

161. Fucharoen S, Shimizu K, Fukumaki Y. A novel C-T transition within the distal CCAAT motif of the G gamma-globin gene in the Japanese HPFH: implication of factor binding in elevated fetal globin expression. **Nucleic Acids Res** 1990; 18(17): 5245-53.

162. Miles KL, Norwich JT, Martinson JJ, Clegg JB. Polymerase chain reaction protocols for alpha globin haplotype polymorphisms. **Br J Haematol** 2001; 113: 694-8.

163. Fukumaki Y, Fucharoen S. Generation and spread of globin mutations in population: β-thalassemia in Asian countries. In: Kimura M, Takahata N, editors. **New Aspects of the Genetics of Molecular Evolution**. Berlin: Springer-Verlag; 1991. p. 153-76.

164. Singsanan S, Fucharoen G, Savongsy O, Sanchaisuriya K, Fucharoen S. Molecular characterization and origins of Hb Constant Spring and Hb Pakse in Southeast Asian populations. **Ann Hematol** 2007; 86(9): 665-9.

165. Boonsa S, Sanchaisuriya K, Fucharoen G, Wiangnon S, Jetsrisuparb A, Fucharoen S. The diverse molecular basis and hematologic features of Hb H and AEBart's diseases in northeast Thailand. **Acta Haematol** 2004; 111: 149-54.

166. Nuntakarn L, Fucharoen S, Fucharoen G, Sanchaisuriya K, Jetsrisuparb A, Wiangnon S. Molecular, hematological and clinical aspects of thalassemia major and thalassemia intermedia associated with Hb E-β-thalassemia in northeast Thailand. **Blood Cells Mol Dis** 2009; 42: 32-5.

167. Tanphaichitr VS, Viprakasit V, Veerakul G, Sanpakit K, Tientadakul P. Homozygous Hemoglobin Tak causes symptomatic secondary polycythemia in a Thai boy. **J Pediatr Hematol Oncol** 2003; 25(3): 261-5.

168. Sanchaisuriya K, Fucharoen G, Sae-ung N, Jetsrisuparb A, Fucharoen S. Molecular and hematologic features of Hemoglobin E heterozygotes with different forms of α-thalassemia in Thailand. **Ann Hematol** 2003; 82(10): 612-6.

169. Smid M, Vassallo A, Lagona F, Valsecchi L, Maniscalco L, Danti L, et al. Quantitative analysis of fetal DNA in maternal plasma in pathological conditions associated with placental abnormalities. **Ann N Y Acad Sci** 2001; 945: 132-7.

170. Zhong XY, Holzgreve W, Hahn S. The levels of circulatory cell free fetal DNA in maternal plasma are elevated prior to the onset of preeclamsia. **Hypertens Pregnancy** 2002; 21(1): 77-83.

171. Li D, Liao C, Xie X, Zhong H, Li J. Four cases of Hb Q-H disease found in Southern China. **Hemoglobin** 2007; 31: 109-11.

172. Tan JAMA, Tay JSH, Wong YC, Kham SKY, Aziz NBA, Teo SH, et al. Molecular analysis of Hb Q-H and HbQ-Hb E in a Singaporean family, Southeast Asian. **J Trop Med Public Health** 1995; 850: 415-9.

173. Zheng W, Liu Y, Chen D, Rong K, Ge Y, Gong C, et al. Complex interaction of Hb Q-Thailand and Hb E with αo-thalassemia and hereditary persistence of fetal hemoglobin in a Chinese family. **Ann Hematol** 2010; 89 (9): 883-8.

174. Schrier SL, Bunyaratvej A, Khuhapinant A, Fucharoen S, Alijurt M, Snyder LM, et al. The unusual pathobiology of hemoglobin Constant Spring red blood cells. **Blood** 1997; 89: 1762-9.

175. Liebhaber SA, Russell JE. Expression and developmental control of the human α-globin gene cluster. **Ann NY Acad Sci** 1998; 850: 54-63.

176. Lin M, Wu JR, Yang LY. Hb Q-H disease: two cases in a Cantonese family. **Blood Cells Mol Dis** 2008; 41: 259-60.

177. Li D, Liao C, Li J, Xie X, Zhong H. Association of Hb Q-Thailand with heterozygous Hb E in a Chinese patient. **Hemoglobin** 2008; 32: 319-21.

178. Fucharoen S, Fucharoen G, Sae-ung N, Sanchaisuriya K. Thalassemia intermedia associated with the Hb Constant Spring EE Bart's disease in pregnancy: a molecular and hematological analysis. **Blood Cells Mol Dis** 2007; 39: 195-8.

179. Fucharoen S, Winichagoon P, Sirithanaratkul N, Chowthaworn J, Pootrakul P. α And β thalassemia in Thailand. **Ann NY Acad Sci** 1998; 850: 412-4.

180. Liao C, Li J, Li D. Association of β-thalassemia and Hb Q-Thailand resulting in a normal Hb A2 value. **Hemoglobin** 2008; 32: 505-8.

181. Siriratmanawong N, Fucharoen G, Sanchaisuriya K, Ratanasiri T, Fucharoen S. Rapid and simultaneous detection of β-thalassemia and α-thalassemia 1 (SEA type) in prenatal diagnosis of complex thalassemia syndrome. **Clin Biochem** 2001; 34: 377-80.

182. Minnichi V, Hill RJ, Khuri PD, Anderson ME. Hemoglobin Hope: A Beta Chain Variant. **Blood** 1965; 25: 830-8.

183. Ingle J, Adewoye A, Dewan R, Okoli M, Rollins L, Eung SH, et al. Hb Hope [beta136(H14)Gly-->Asp(GGT-->GAT)]: interactions with Hb S [beta6(A3) Glu --> Val (GAG-->GTG)], other variant hemoglobins and thalassemia. **Hemoglobin** 2004; 28(4): 277-85.

184. Svasti S, Yodsowon B, Sriphanich R, Winichagoon P, Boonkhan P, Suwanban T, et al. Association of Hb Hope [beta136(H14)Gly-->Asp] and Hb H disease. **Hemoglobin** 2001; 25(4): 429-35.

185. Sura T. Haemoglobin Hope in a northern Thai family: first identification of homozygous haemoglobin Hope associated with haemoglobin H disease. **Eur J Haematol** 2007; 79(3): 251-4.

186. Pillers DA. Hb Hope [beta 136(H14) Gly--Asp] and Hb E [beta26(B8)Glu--Lys]: compound heterozygosity in Thai Mien family. **Hemoglobin** 1992; 16: 81-4.

187. Pearson TC. Diagnosis and classification of erythrocytoses and thrombocytoses. **Baillieres Clin Haematol** 1998; 11: 695-720.

188. Charoenkwan P, Thanarattanakorn P, Chaovaluksakul S, Sittipreechacharn S, Saetung R, Sanguansermsri T. Hematological and molecular characterization of beta thalassemia/ Hb Tak compound heterozygote. **Southeast Asian J Trop Med Public Health** 2003; 34: 415-9.

189. Teawtrakul N, Sirijirachai C, Changsung G, Fucharoen G. Compound heterozygous Hb Tak/Hb E causes secondary erythrocytosis in a Thai boy. **Hemoglobin** 2010; 34: 165-8.

190. Papassotiriou I, Traeger-Synodinos J, Marden MC, Kister J, Liapi D, Prome D, et al. The homozygous state for Hb Crete [b129(H7) Ala-Pro] is associated with a complex phenotype including erythrocytosis and functional anemia. **Blood Cells Mol Dis** 2005; 34: 229-34.

191. Huisman THJ. Hb E and α-thalassemia; variability in the assembly of β^E chain containing tetramers. **Hemoglobin** 1997; 21(3): 227-36.

192. Huisman THJ, Carver MFH, Efremov GD. **A Syllabus of Human Hemoglobin Variants**. 2 ed. USA: The Sickle Cell Anemia Foundation; 1998.

193. Savongsy O, Fucharoen S, Fucharoen G, Sanchaisuriya K, Sae-ung N. Thalassemia and hemoglobinopathies in pregnant Lao women: carrier screening, prevalence and molecular basis. **Ann Hematol** 2008; 87(8): 647-54.

194. Kossover CL, Eckman JR, Young AN. Compound heterozygosity for Hemoglobin C and Hemoglobin Korle-Bu. **Lab Hematol** 2008; 14(3): 30-4.

195. Honig GR, Adams JGI. **Human hemoglobin genetics**. Berlin: Springer-Verlag; 1986.

196. Rochette J, Barnetson R, Kiger L, Kister J, Littlewood TJ, Webster R, et al. Association of a novel high oxygen affinity haemoglobin variant with delta beta thalassaemia. **Br J Haematol** 1994; 86(1): 118-24.

APPENDICES

APPENDIX A

Research presentations

1. **สานิตา สิงห์สนั่น**, กุลนภา ฟู่เจริญ, กนกวรรณ แสนไชยสุริยา, ณัฐยา แซ่อึ้ง, สุพรรณ ฟู่เจริญ. Interactions of Hb Q-Thailand with various hemoglobinopathies: molecular and hemoglobin analysis. **การประชุมวิชาการของสำนักงานคณะกรรมการการอุดมศึกษา ครั้งที่ 1: เครือข่ายกลยุทธ์เพื่อการพัฒนาบุคลากรมหาวิทยาลัย.** โรงแรม แอมบาสเดอร์ซิตี้ จอมเทียน จ.ชลบุรี, 5-7 กันยายน 2551. (poster presentation)

2. **Singsanan S**, Fucharoen G, Sanchaisuriya K, Sae-ung N, Fucharoen S. Interactions of Hb Q-Thailand with various hemoglobinopathies: molecular and hemoglobin analysis. **The 1st Conference of Biomedical Sciences, A novel concurrence research.** 8th floor, Faculty of Associated Medical Sciences, Khon Kaen University, 14 July 2010. (Poster presentation)

3. **สานิตา สิงห์สนั่น**, กุลนภา ฟู่เจริญ, สุพรรณ ฟู่เจริญ. Hb Hope [β136(H4)Gly-Asp] related disorders in Thailand: origin, interactions with various hemoglobinopathies and diagnostic aspects. **การประชุมวิชาการของสำนักงานคณะกรรมการการอุดมศึกษา ครั้งที่ 3 : เครือข่ายกลยุทธ์เพื่อการพัฒนาบุคลากรมหาวิทยาลัย.** โรงแรมรอยัล คลิฟ บีช รีสอร์ท, พัทยา จังหวัดชลบุรี. 9 – 11 กันยายน 2553. (poster presentation)

4. **Singsanan S**, Fucharoen G, Fucharoen S. Hb Hope [β136(H4)Gly-Asp] related disorders in Thailand: origin, interactions with various hemoglobinopathies and diagnostic aspects. Paper presented at **The 33rd World Congress of the International Society of Hematology**, 10-13 October 2010, ICC Jerusalem International Convention Center 1 Shazar, Jerusalem, Israel. (Poster presentation)


การประชุมวิชาการของสำนักงานคณะกรรมการการอุดมศึกษา ครั้งที่ 1
เครือข่ายเชิงกลยุทธ์เพื่อการพัฒนาบุคลากรมหาวิทยาลัย
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PCI - 49

Interactions of Hb Q-Thailand with various hemoglobinopathies: molecular and hemoglobin analysis

nita Singsanan[1,2], Goonnapa Fucharoen[1], Supan Fucharoen[1], Supawadee Yamsri[1,2], Kanokwan Sanchaisuriya[1] and Nattaya Sae-ung[1]
Centre for Research and Development of Medical Diagnostic Laboratory, Faculty of Associated Medical Sciences
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tives

To describe the molecular characteristics and hemoglobin profiles associated with emia syndromes caused by interactions of Hb Q-Thailand with various globinopathies in Thai patients encountered at our laboratory.

ods

Study was conducted on 39 unrelated Thai individuals with abnormal Hb resembling Thailand observed on our routine Hb analysis using automated HPLC and capillary phoresis systems. Hematological analysis was performed using automated blood cell rs. The $\alpha^{Q\text{-Thailand}}$ mutation and the linked ($-\alpha^{4.2}$) deletion were identified by a multiplex pecific PCR assay.

s

ig. 1 demonstrated results of PCR analysis of the Hb Q-T mutation [α74(EF3) Asp- nd the linked ($-\alpha^{4.2}$) which was confirmed in all cases and ten genotypes were observed e groups. The first group included 16 Hb Q-T heterozygotes, 1 compound Hb Q-T/α^{+}-emia ($-\alpha^{3.7}$), 2 Hb Q-T/CS disease, 4 Hb Q-T/H disease and 1 homozygous Hb Q-T. In oup, the average levels of Hb Q-T were 29.2%, 34.7%, 49.2-49.3%, 77.8% and 82.3%, vely. In the second group, Hb Q-T was found in association with Hb E [β26(B8)Glu- 7 double heterozygotes for Hb Q-T/Hb E, 2 Hb Q-T/α^{+}-thalassemia ($-\alpha^{3.7}$)/Hb E zygote, 3 heterozygous Hb Q-T/homzygous Hb E and 1 Hb Q-T/CS/homozygous Hb ddition to the Hb E ($\alpha^{A}{}_2\beta^{E}{}_2$) and Hb Q-T ($\alpha^{Q}{}_2\beta^{A}{}_2$) fractions, a small peak with slower on time was clearly observed in the two former genotypes, most likely the Hb QE d from the ($\alpha^{Q}{}_2\beta^{E}{}_2$) tetrameric assembly. The remaining two cases of the third group und to be a double heterozygote for Hb Q-T/β^{0}-thalassemia. The levels of Hb Q-T 2), Hb A_2 ($\alpha^{A}{}_2\delta_2$) and Hb Q-A_2 ($\alpha^{Q}{}_2\delta_2$) were 13.8-16.6%, 4.9-5.3% and 1-2.6%, tively.

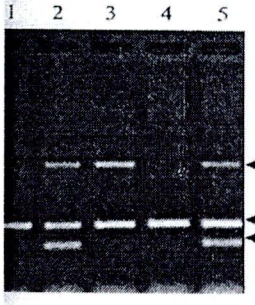

Fig.1 A representative agarose gel electrophoresis of the multiplex allele-specific PCR. M : λ /Hind III size markers, lane 1 and 4: normal, lane 2 and 5: positive for Hb Q-Thailand and lane 3: positive for $-\alpha^{4.2}$.

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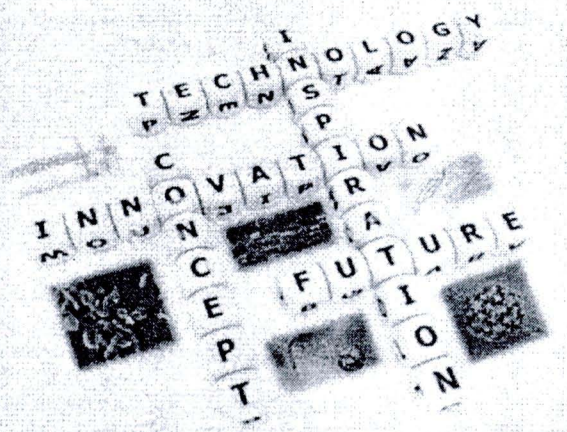

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Interactions of Hb Q-Thailand with various hemoglobinopathies: molecular and hemoglobin analysis

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ABSTRACT

To describe the molecular characteristics and hemoglobin profiles associated with thalassemia syndromes caused by interactions of Hb Q-Thailand with various hemoglobinopathies in Thai patients encountered at our laboratory. The study was conducted on 56 unrelated Thai individuals with abnormal Hb resembling Hb Q-Thailand observed on our routine Hb analysis using automated HPLC and capillary electrophoresis systems. Hematological analysis was performed using automated blood cell counters. The $\alpha^{Q\text{-}Thailand}$ mutation and the linked ($-\alpha^{4.2}$) deletion were identified by a multiplex allele specific PCR assay. The molecular diagnosis of the Hb Q-T mutation [α74(EF3) Asp-His] and the linked ($-\alpha^{4.2}$) was confirmed in all cases and ten genotypes were observed in three groups. The first group included 28 Hb Q-T heterozygotes, 1 compound Hb Q-T/□$^{+}$-thalassemia ($-□^{3.7}$), 2 Hb Q-T/CS disease, 6 Hb Q-T/H disease and 1 homozygous Hb Q-T. In this group, the average levels of Hb Q-T were 29.8%, 34.7%, 49.2-49.3%, 79.4% and 82.3%, repectively. In the second group, Hb Q-T was found in association with Hb E [□ 26(B8)Glu-Lys]; 9 double heterozygotes for Hb Q-T/Hb E, 3 Hb Q-T/□$^{+}$-thalassemia ($-□^{3.7}$)/Hb E heterozygote, 3 heterozygous Hb Q-T/homzygous Hb E and 1 Hb Q-T/CS/homozygous Hb E. In addition to the Hb E ($□^{A}_{□}□^{E}_{□}$) and Hb Q-T ($□^{Q}_{□}□^{A}_{□}$) fractions, a small peak with slower retention time was clearly observed in the two former genotypes, most likely the Hb QE resulted from the ($□^{Q}_{□}□^{E}_{□}$) tetrameric assembly. The remaining two cases of the third group was found to be a double heterozygote for Hb Q-T/□^{0}-thalassemia. The levels of Hb Q-T ($□^{Q}_{□}□^{A}_{□}$), Hb $A_{□}$ ($□^{A}_{□}□_{□}$) and Hb Q-$A_{□}$ ($□^{Q}_{□}□_{□}$) were 13.8-16.6%, 4.9-5.3% and 1-2.6%, respectively. Hb Q-T may not be uncommon in Thailand and probably in other Southeast Asian countries as previously noted. Interaction of this Hb Q-T with other forms of thalassemia and hemoglobinopathies is common and could lead to complex thalassemia syndromes with various phenotypic features. Accurate diagnosis of these syndromes is essential for providing appropriate genetic counseling which requires both hematologic and molecular analyses.

Program & Abstracts

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Hb hope related disorders in Thailand: origin, interactions with various hemoglobinopathies and diagnostic aspects

Sanita Singsanan[1,2], Goonnapa Fucharoen[2], Supan Fucharoen[2*]
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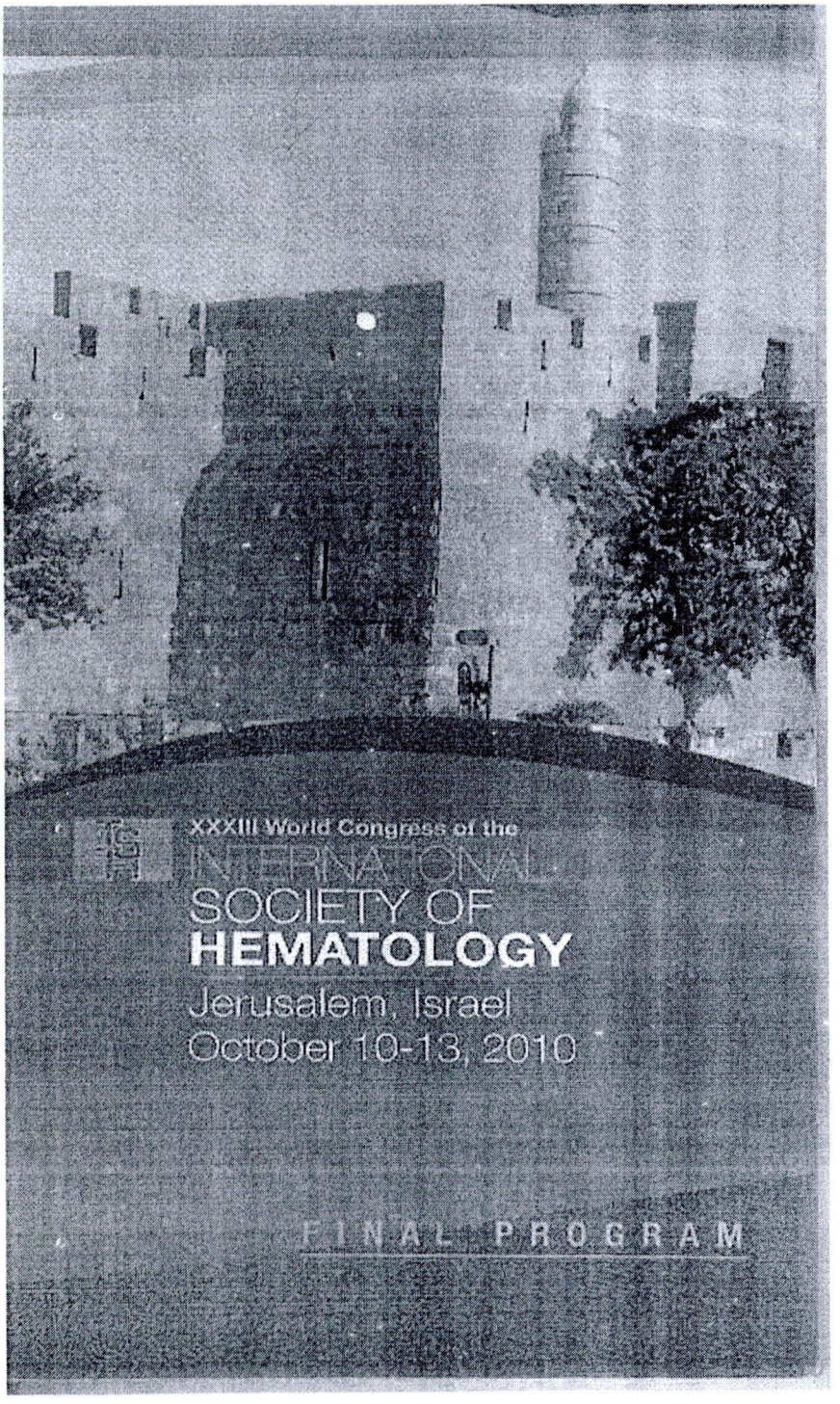
Introduction: Hemoglobin (Hb) Hope [β136(H14)Gly→Asp] is mildly unstable β-globin chain variant originally described in an African-American family and has been found in many ethnic backgrounds.
Objectives : To describe the molecular and hematological profiles associated with syndromes caused by interactions of this variant with various hemoglobinopathies in Thai patients.
Results: Nine different genotypes were observed which were classified into 4 groups. **Group I** included 25 Hb Hope heterozygotes with relatively normal hematological features and an average level of 42.2 % Hb Hope. **Group II** included those with Hb Hope and α-thalassemia including 1 double heterozygous Hb Hope/α-thalassemia 2, 1 Hb Hope/Hb Constant Spring, 3 Hb Hope/α-thalassemia 1 and 1 Hb Hope/Hb H disease. The average levels of Hb Hope were 48.5 %, 43.1 %, 14.3-29.6 % and 21.6 %, respectively. Minute amounts of Hb Bart's but not Hb H was observed in a patient with Hb Hope/Hb H-disease. **Group III** included two compound Hb Hope/β^0-thalassemia, 1 Hb Hope/β^0-thalassemia/α-thalassemia 2 and 1 Hb Hope/β^0-thalassemia/α-thalassemia 1. In this group, the average levels of Hb Hope were 90.1 %, 73.1 % and 78.5 %, respectively. Hb A_2 was elevated in all cases. **Group IV** included 6 compound Hb Hope/Hb E heterozygotes with the average levels of 66.1 % Hb Hope and 27.8 % Hb E. Haplotype analysis demonstrated that all these Thai β^{Hope} genes were associated with the same haplotype, (+ - - - - + +), indicating likely a single origin of this variant in Thai population.
Discussion and Conclusion: Although Hb Hope is identified on both HPLC and capillary electrophoresis and clinically innocuous, differentiation from those of clinically relevant Hb variants would require DNA-based diagnostics.

Keywords: Hb Hope, Hemoglobinophathies, PCR

Poster Presentation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08:00-17:00 **Poster Area**

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165 **SICKLE CELL DISEASE-ERYTHROCYTE OXIDATIVE STRESS-IMPAIRED DEFORMABILITY AND CRISIS**
J. Rifkind, V. Barodka, E. Nagababu, J. Mohanty, D. Nyhan, D. Berkowirz, J. Strouse, *Baltimore, MD, USA*

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APPENDIX B

Research publications

1. Siriratmanawong N, Chansri W, **Singsanan S**, Fucharoen G, Fucharoen S. Complex interaction of Hb E [beta26(B8)Glu-->Lys], Hb Korle-Bu [beta73(E17)Asp-->Asn] and a deletional alpha-thalassemia-1 in pregnancy. **Hemoglobin** 2009; 33(6): 507-14.
2. **Singsanan S**, Karnpean R, Fucharoen G, Sanchaisuriya K, Sae-Ung N, Fucharoen S. Hemoglobin Q-Thailand related disorders: origin, molecular, hematological and diagnostic aspects. **Blood Cells Mol Dis** 2010; 45(3): 210-4.
3. Prakobkaew N, **Singsanan S**, Fucharoen G, Surapot S, Fucharoen S. Secondary Erythrocytosis Caused by Hemoglobin Tak/$(\delta\beta)^0$-Thalassemia Syndrome. **Acta Haematol** 2010; 124(2): 115-119.
4. **Singsanan S**, Srivorakun H, Fucharoen G, Puangplruk R, Fucharoen S. Hemoglobin Phimai [β72(E16)Ser→Thr]: a novel β-globin structural variant found in association with Hb Constant Spring in pregnancy. **Hemoglobin** 2010. (in press)
5. **Singsanan S**, Fucharoen G, Fucharoen S. Hb Hope related disorders in Thailand: origin, interactions with various hemoglobinopathies and diagnostic aspects. (manuscript in preparation)
6. **Singsanan S**, Srivorakul H, Fucharoen G, Sanchaisuriya K, Sae-ung N, Fucharoen S. The levels of total glyceraldehyde-3-phosphate dehydrogenase (GAPDH) DNA in maternal plasma from first and second trimester of pregnancies with Hb Bart's hydrop fetalis. (manuscript in preparation)

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SHORT COMMUNICATION

COMPLEX INTERACTION OF Hb E [β26(B8)Glu→Lys], Hb KORLE-BU [β73(E17)Asp→Asn] AND A DELETIONAL α-THALASSEMIA-1 IN PREGNANCY

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□ *A pregnant Thai woman with mild hypochromic microcytic anemia caused by α- and β- globin defects is described. The proband was a 26-year-old pregnant woman discovered through our ongoing thalassemia screening program. Initial hemoglobin (Hb) high performance liquid chromatography (HPLC) analysis revealed a homozygosity for an unknown variant at the D window, inconsistent with results of family analyses. Further Hb analysis using automated capillary zone electrophoresis identified that the proband was in fact a compound heterozygote for Hb E [β26(B8)Glu→Lys, GAG>AAG] and another β chain variant. DNA analysis demonstrated that she carried the Hb Korle-Bu mutation [β73(E17)Asp→Asn (GAT>AAT)] in trans to the Hb E and an α-thalassemia-1 (α-thal-1) with the Southeast Asian (– $-^{SEA}$) deletion. Family studies identified that her father and sister were double heterozygotes for Hb Korle-Bu and α-thal-1, whereas her mother was a double heterozygote for Hb E/Hb Constant Spring [Hb CS; α142, Term→Gln (TAA>CAA in α2)]. The genotype-phenotype relationship observed in this Thai family with complex hemoglobinopathies and methods for characterization are presented.*

Keywords Hb E, Hb Korle-Bu, α-Thalassemia (α-thal)

Thalassemia and hemoglobinopathies are the most common genetic disorders in Southeast Asia. In Thailand, for example, the frequency of α-thalassemia

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(α-thal) reaches 20–30% and that of β-thal varies between 3–9%. The average prevalence of Hb E trait is estimated at 25–30% but exceeding 50% in some minority groups of northeast Thailand, and that of Hb Constant Spring [Hb CS: α142, Term→Gln (TAA>CAA in α2)] is 1–8%. Other abnormal Hbs caused by both α and β chain variants are occasionally reported (1,2). In these areas, cases with complex phenotypes caused by the interaction of mutations affecting both α- and β-globin genes, requiring multiple methods for accurate diagnosis are not uncommon (3–6). Using a combination of hemoglobin (Hb) high performance liquid chromatography (HPLC) analysis, capillary zone electrophoresis and DNA analysis, we describe here a hitherto undescribed condition found in a pregnant Thai woman carrying Hb E [β26(B8)Glu→Lys, *GAG>AAG*], Hb Korle-Bu [β73(E17)Asp→Asn (*GAT>AAT*)] (7) and a deletional α-thal-1. Hematologic data of the patient was compared with those of her father and her sister who carried Hb Korle-Bu and α-thal-1 but not Hb E, and the mother who was a double heterozygote for Hb E and Hb CS as well as another pregnant Thai woman with Hb E, Hb Korle-Bu and α-thal-2 whom we described in an earlier report (8).

Ethical approval for the study protocol was obtained from our Institutional Review Board (iRB) at Khon Kaen University (HE481115) and informed consent was obtained. The proband was a 26-year-old pregnant woman who presented at our ongoing thalassemia screening program at the Health Promoting Centre Region 8, Nakornsawan, Thailand, with mild hypochromic microcytic anemia. She had positive results with both osmotic fragility and dichlorophenolindophenol (DCIP) tests, a combined screening test for thalassemia and hemoglobinopathies generally used in the prevention and control program of thalassemia in Thailand (9,10).

Blood specimens from her family members including the father, mother and older sister were also available for testing. As shown in Table 1, the proband had the following values: Hb 10.3 g/dL, PCV 0.298 L/L, MCV 66.1 fL, MCH 22.8 pg, MCHC 34.6 g/dL and RDW 14.9%. G-6-PD deficiency and iron deficiency were excluded. Peripheral blood film examination showed slight anisocytosis and hypochromic red cells with target cells. Hemoglobin analysis using automated HPLC (VARIANT™; Bio-Rad Laboratories, Hercules, CA, USA) revealed no Hb A but a single peak of an abnormal Hb at the D window with the amount of 88.2% (Figure 1). She was therefore initially diagnosed as a homozygous Hb D-Punjab [β121(GH4)Glu→Gln, *GAA>CAA*] or Hb Tak [β147 (+AC)], the two variants commonly found in Thai population or other β chain variants (11,12).

Inconsistencies with this diagnosis were a positive result obtained with a DCIP test for Hb E screening and a result of family analysis which identified the same variant only in the father but not in the mother. In addition, a negative result with a multiplex allele-specific polymerase chain reaction

TABLE 1 Hematological Data and Genotypes of the Proband, Her Family and the Previous Case (8)

Parameters	Father	Mother	Sister	Proband	Previous case
Sex-Age (years)	M-67	F-63	F-32	F-26	F-19
Osmotic fragility	positive	negative	positive	positive	positive
DCIP	negative	positive	negative	positive	positive
Hb (g/dL)	11.2	11.8	12.3	10.3	12.2
PCV (L/L)	0.335	0.362	0.376	0.298	0.360
MCV (fL)	76.5	80.1	63.6	66.1	73.2
MCH (pg)	25.6	25.9	20.8	22.8	24.8
MCHC (g/dL)	33.4	32.3	32.7	34.6	33.9
RDW (%)	19.3	13.7	15.7	14.9	15.9
Hb A (%)	51.9	73.5	51.9	–	–
Hb Korle-Bu (%)	45.9	–	45.4	80.0	69.3
Hb E (%)	–	23.5	–	16.4	22.4[a]
Hb A_2 (%)	2.2	3.0	2.7	3.6	–
α Genotype	$\alpha\alpha/--^{SEA}$	$\alpha\alpha/\alpha^{CS}\alpha$	$\alpha\alpha/--^{SEA}$	$\alpha\alpha/--^{SEA}$	$\alpha\alpha/-\alpha^{3.7}$
β Genotype	$\beta^A/\beta^{Korle\text{-}Bu}$	β^A/β^E	$\beta^A/\beta^{Korle\text{-}Bu}$	$\beta^E/\beta^{Korle\text{-}Bu}$	$\beta^E/\beta^{Korle\text{-}Bu}$
β Haplotype[b]	β^A [+ – – – – + –] $\beta^{Korle\text{-}Bu}$ [– + – + + + –]	β^A [– + – + + + –] β^E [– + – + + + –]	β^A [– + – + + + –] $\beta^{Korle\text{-}Bu}$ [– + – + + + –]	β^E [– + – + + + –] $\beta^{Korle\text{-}Bu}$ [– + – + + + –]	β^E [– + – + + + –] $\beta^{Korle\text{-}Bu}$ [– + – + + + –]

[a]Including Hb A_2 as determined by the electrophoretic elution technique.

[b]Including seven polymorphic sites: *Hin*dIII–ε; *Hin*dIII–$^A\gamma$; *Hin*dIII–$^G\gamma$; *Hin*cII–$\psi\beta$; *Hin*cII–3′$\psi\beta$; *Ava*II–β; *Bam*HI–3′β.

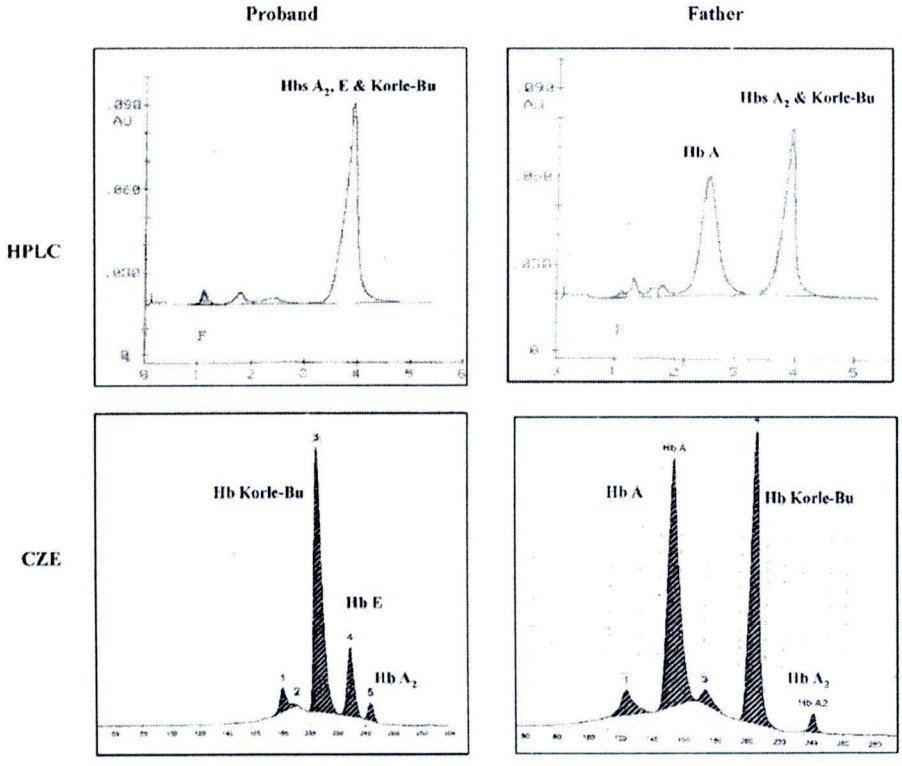

FIGURE 1 Hemoglobin analyses of the proband and her father using automated HPLC and capillary zone electrophoresis. The separating profiles of Hb A_2, Hb E and Hb Korle-Bu are indicated.

(PCR) for Hb D-Punjab, Hb Tak and Hb S [β6(A3)Glu→Val, GAG>GTG] mutations was obtained (13). Further Hb analyses of the proband using capillary zone electrophoresis (Capillarys 2; Sebia, Lisses. France), however, clearly demonstrated Hb A_2 (3.6%), Hb E (16.4%) and an abnormal Hb (80.0%) migrating separately between Hb A and Hb E (Figure 1). This Hb analysis system can report Hb A_2 in the presence of Hb E. This abnormal Hb (45.9%) and Hb A_2 (2.2%) were also observed in addition to the Hb A (51.9%) in her father, whereas her mother was found to be a heterozygous Hb E. As with her father, this Hb analysis identified that her sister was heterozygous for the same variant. As shown in Table 1, the proband and her mother had relatively lower Hb E levels (16.4 and 23.5%, respectively) when compared to those described for Hb E heterozygotes, the data indicating a possibility of co-inheritance of α-thal (14). Therefore, α-globin genotyping by PCR (15–17) was carried out for all family members. With this analysis, we identified the Southeast Asian deletional α-thal-1 in the

proband, her father and her sister but not in her mother who was found to carry the Hb CS mutation.

Further DNA analysis using allele-specific PCR for abnormal Hbs found in Thailand identified that the Hb variant segregating in this family was Hb Korle-Bu caused by the G>A mutation at codon 73 of the β-globin gene that leads to a substitution of asparagine for aspartic acid (8). In the present family, as expected, the Hb Korle-Bu mutation was identified in the proband, her father and her sister but not in her mother (Figure 2). Therefore, with these analyses we were able to conclude that the proband carried Hb E, Hb Korle-Bu and α-thal-1, whereas her father and her sister had Hb Korle-Bu and α-thal-1, both of which are novel conditions. Her mother was a double heterozygote for Hb E and Hb CS. In Table 1, the hematological parameters of the proband and her family members were compared with

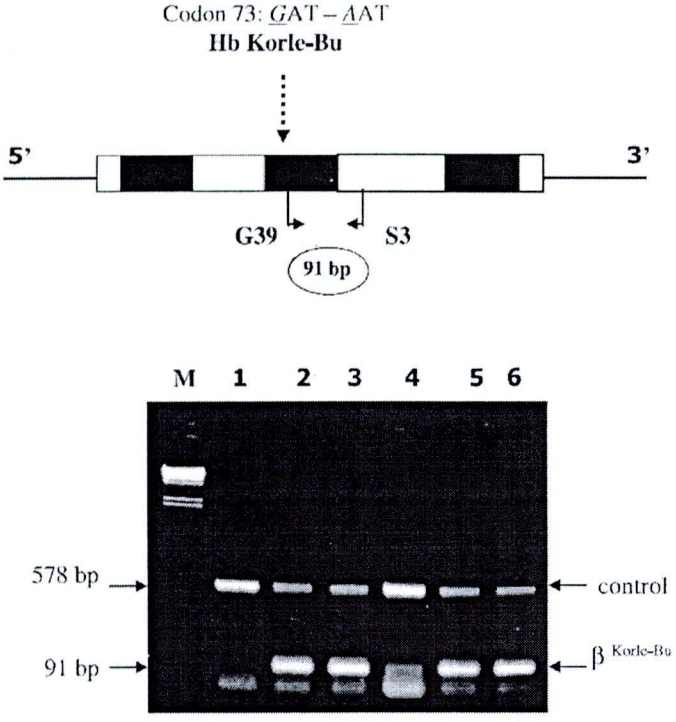

FIGURE 2 Identification of the Hb Korle-Bu mutation by allele-specific PCR. The locations and orientations of primers G39 and S3 and the size of amplified fragments are depicted. The 578 bp fragment generated from the $^{G}\gamma$-globin gene promoter is an internal control fragment, whereas the 91 bp fragment derived from primers G39 and S3 is the $\beta^{Korle\text{-}Bu}$-specific fragment (8). M: λ/*Hin*dIII size markers; lanes 1 and 2: normal and positive controls for Hb Korle-Bu; lanes 3–6: the father, the mother, the sister and the proband, respectively.

those of another pregnant Thai woman with Hb E/Hb Korle-Bu/α-thal-2 (3.7 kb deletion) described previously (8). As shown in Table 1, the proband with Hb E/Hb Korle-Bu/α-thal-1 clearly had more pronounced hypochromic microcytic anemia with the lower proportion of Hb E (16.4%). It is also noteworthy that in the proband the level of Hb Korle-Bu (80.0%) is much greater than that of Hb E (16.4%). This likely indicates that as the availability of α chain is decreased in α-thal-1, the formation of Hb Korle-Bu is favored over the formation of Hb E (18).

Hb Korle-Bu is a non pathological β chain variant found in the people of several countries of West Africa, Guadeloupe, Mexico, Ivory Coast, Spain and the rural district of Jamaica (19). In Southeast Asia, Hb Korle-Bu has only been reported in Thailand and Lao People's Democratic Republic where it has been observed associated to a single β-globin gene haplotype, [− + − + + − +] (8,20). As shown in Table 1, we found that the same β-globin haplotype was associated with the $\beta^{\text{Korle-Bu}}$ in the family studied here. All these Thai and Laotian families with Hb Korle-Bu are unrelated and appear to have no historical link with Africa. Although the β-globin haplotype for the African $\beta^{\text{Korle-Bu}}$ has not been reported, our data indicates the same origin for this mutation in the Southeast Asian population.

Even though a heterozygous form of this Hb variant is clinically asymptomatic, compound heterozygous states with other hemoglobinopathies and thalassemias can cause serious conditions. It is therefore important to distinguish this Hb variant from other common carriers with less or no clinical significance. Interaction of Hb Korle-Bu with Hb C [β6(A3)Glu→Lys, *GAG>AAG*] can cause moderate chronic hemolytic anemia with acceleration of crystal formation (21,22).

Association of Hb Korle-Bu with Hb E and α-thal-2 resulted in a mild anemia. We report for the first time the association of this Hb variant with other common hemoglobinopathies in Southeast Asia; the Hb E and α-thal-1 (SEA deletion) in a pregnant Thai woman. As shown in this study, the proband presented with moderate hypochromic microcytosis with reduced MCV and MCH and Hb levels. Her father and sister having double heterozygosities for Hb Korle-Bu and a deletional α-thal-1 presented with milder hypochromic microcytosis with reduced MCV and MCH and Hb levels (Table 1). Her mother, a double heterozygote for Hb E and Hb CS presented with normochromic normocytosis. Differential diagnoses of these globin gene interactions are therefore important for providing appropriate genetic counseling for the patient and family members.

Diagnosis of Hb Korle-Bu may be problematic in a routine investigation when found in association with other hemoglobinopathies as shown in the presented case. Hb Korle-Bu is not separated from Hb E and Hb A_2 on HPLC analysis, although we have found that the capillary electrophoresis system could help in this separation (Figure 1). However, the final diagnosis

of the case was only possible after DNA analysis. Our study further confirms that for geographical areas where both thalassemias and hemoglobinopathies are prevalent such as Southeast Asia, complex thalassemia syndromes may result from the interaction of mutations affecting both α- and β-globin genes loci with a spectrum of clinical manifestations. It is therefore important to understand and distinguish these gene-gene interactions to be able to provide appropriate genetic counseling. The use of combined methods including HPLC, capillary electrophoresis and DNA analysis, should prove useful in a diagnosis of hemoglobinopathies in those parts of the region where thalassemias and abnormal Hbs are prevalent.

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REFERENCES

1. Fucharoen S, Winichagoon P. Hemoglobinopathies in Southeast Asia. *Hemoglobin.* 1987;11(1):65–88.
2. Svasti J, Srisomsap C, Winichagoon P, Fucharoen S. Detection and structural analysis of abnormal hemoglobins found in Thailand. *Southeast Asian J Trop Med Public Health.* 1999;30(suppl. 2):88–93.
3. Fucharoen S, Ayukarn K, Sanchaisuriya K, Fucharoen G. Atypical Hemoglobin H disease in a Thai patient resulting from a combination of α-thalassemia 1 and Hemoglobin Constant Spring with Hemoglobin J Bangkok heterozygosity. *Eur J Haematol.* 2001;66(5):312–316.
4. Fucharoen S, Changtrakun Y, Ratanasiri T, Fucharoen G, Sanchaisuriya K. Complex interaction of Hb Hekinan [α27(B8)Glu→Asp] and Hb E [β26(B8)Glu→Lys] with a deletional α-thalassemia 1 in a Thai family. *Eur J Haematol.* 2003;70(5):304–309.
5. Jetsrisuparb A, Sanchaisuriya K, Fucharoen G, Fucharoen S, Wiangnon S, Komwilaisak P. Triple heterozygosity of a hemoglobin variant: Hemoglobin Pyrgos with other hemoglobinopathies. *Int J Hematol.* 2002;75(1):35–39.
6. Fucharoen S, Chunpanich S, Sanchaisuriya K, Fucharoen G, Fucharoen S. Thalassemia intermedia associated with complex interaction of Hb Beijing [α16(A14)Lys→Asn] and Hb E [β26(B8)Glu→Lys] with a deletional α-thalassemia-1 in a Thai family. *Hemoglobin.* 2005;29(1):77–83.
7. Konotey-Ahulu FID, Gallo E, Lehmann H, Ringelhann B. Haemoglobin Korle-Bu (β73) aspartic acid-asparagine) showing one of the two amino acid substitutions of Haemoglobin C Harlem. *J Med Genet.* 1968;5(2):107–111.
8. Changtrakun Y, Fucharoen S, Ayukarn K, Siriratmanawong N, Fucharoen G, Sanchaisuriya K. Compound heterozygosity for Hb Korle-Bu (β73; Asp→Asn) and Hb E (β26; Glu→Lys) with a 3.7 kb deletional α-thalassemia in Thai patients. *Ann Hematol.* 2002;81(7):389–393.
9. Fucharoen G, Sanchaisuriya K, Sae-ung N, Dangwibul S, Fucharoen S. A simplified screening strategy for thalassaemia and hemoglobin E in rural communities in south-east Asia. *Bull World Health Org.* 2004;82(5):364–372.
10. Sanchaisuriya K, Fucharoen S, Fucharoen G, et al. A reliable screening protocol for thalassemia and hemoglobinopathies in pregnancy: an alternative approach to electronic blood cell counting. *Am J Clin Pathol.* 2005;123(1):113–118.

11. Fucharoen S, Changtrakun Y, Surapot S, Fucharoen G, Sanchaisuriya K. Molecular and hematological characterization of Hb D-Punjab [β121(GH4)Glu→Gln] in Thailand. *Hemoglobin*. 2002;26(3): 261–269.
12. Tanphaichitr VS, Viprakasit V, Veerakul G, Sanpakit K, Tientadakul P. Homozygous Hemoglobin Tak causes symptomatic secondary polycythemia in a Thai boy. *J Pediatr Hematol Oncol*. 2003; 25(3):261–265.
13. Sanchaisuriya K, Chumpanich S, Fucharoen G, Fucharoen S. Multiplex allele specific PCR assay for differential diagnosis of Hb S, Hb D-Punjab and Hb Tak. *Clin Chim Acta*. 2004;89(1–2):777–781.
14. Sanchaisuriya K, Fucharoen G, Sae-ung N, Jetsrisuparb A, Fucharoen S. Molecular and hematologic features of Hemoglobin E heterozygotes with different forms of α-thalassemia in Thailand. *Ann Hematol*. 2003;82(10):612–616.
15. Fucharoen S, Sanchaisuriya K, Fucharoen G, Panyasai S, Devenish R, Luy L. Interaction of Hemoglobin E and several forms of α-thalassemia in Cambodian families. *Haematologica*. 2003;88(10):1092–1098.
16. Boonsa S, Sanchaisuriya K, Fucharoen G, Wiangnon S, Jetsrisuparb A, Fucharoen S. The diverse molecular basis and hematological features of Hb H and AEBart's diseases in northeast Thailand. *Acta Haematol*. 2004;111(3):149–154.
17. Fucharoen G, Fucharoen S. Rapid and simultaneous non-radioactive method for detecting α-thalassemia 1 (SEA type) and Hb Constant Spring genes. *Eur J Haematol*. 1994;53(3):186–187.
18. Huisman THJ. Hb E and α-thalassemia; variability in the assembly of α_2^E chain containing tetramers. *Hemoglobin*. 1997;21(3):227–236.
19. Huisman THJ, Carver MFH, Efremov GD. *A Syllabus of Human Hemoglobin Variants (second edition)* Augusta: The Sickle Cell Anemia Foundation, 1998 (http://globin.cse.psu.edu).
20. Savongsy O, Fucharoen S, Fucharoen G, Sanchaisuriya K, Sae-ung N. Thalassemia and hemoglobinopathies in pregnant Lao women: carrier screening, prevalence and molecular basis. *Ann Hematol*. 2008;87(8):647–654.
21. Nagel RL, Lin MJ, Witkowska E, Fabry ME, Bestak M, Hirsch E. Compound heterozygosity for Hemoglobins C and Korle-Bu: moderate microcytic anemia and acceleration of crystal formation. *Blood*. 1993;82(6):1907–1912.
22. Kossover CL, Eckman JR, Young AN. Compound heterozygosity for Hemoglobin C and Hemoglobin Korle-Bu. *Lab Hematol*. 2008;14(3):30–34.

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Hemoglobin Q-Thailand related disorders: Origin, molecular, hematological and diagnostic aspects

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ABSTRACT

We describe the molecular and hematological profiles of thalassemia syndromes caused by interactions of hemoglobin (Hb) Q-Thailand [α74(EF3) Asp-His] and various hemoglobinopathies found in 52 unrelated adult Thai subjects. Ten genotypes including several previously undescribed conditions were observed, which were classified into 4 groups. Group I included 26 Hb Q-Thailand heterozygotes and a homozygotous subject. Group II included subjects with Hb Q-Thailand and other α-thalassemia alleles *in trans* including 1 compound Hb Q-Thailand/α^+-thalassemia ($-\alpha^{3.7}$), 2 Hb Q-Thailand/Hb Constant Spring disease and 6 Hb H/Q-Thailand disease. The average levels of Hb Q-Thailand were found to be 29.8%, 82.3%, 34.7%, 49.2–49.3% and 79.4%, respectively. Both Hbs Bart's and H were observed in addition to Hb Q-Thailand in all 6 cases with Hb Q-H disease but not in a homozygous Hb Q-Thailand. Group III included 7 double heterozygotes for Hb Q-Thailand/Hb E, 3 Hb Q-Thailand/Hb E/α^+-thalassemia ($-\alpha^{3.7}$), 3 heterozygous Hb Q-Thailand/homozygous Hb E and 1 triple heterozygote for Hb Q-Thailand/Hb Constant Spring/Hb E. In this group, Hbs E ($\alpha^{A}_2\beta^{E}_2$), Q-Thailand ($\alpha^{QT}_2\beta^{A}_2$) and QE ($\alpha^{QT}_2\beta^{E}_2$) were observed on both HPLC and capillary electrophoresis. The Hb QE, rather than Hb Q-Thailand, was detected in all 3 cases with heterozygous Hb Q-Thailand and homozygous Hb E. The remaining two cases in group 4 were double heterozygotes for Hb Q-Thailand and β^0-thalassemia in which Hb Q-Thailand, elevated Hb A_2 ($\alpha^{A}_2\beta^{A}_2$), and Hb QA_2 ($\alpha^{QT}_2\beta^{A}_2$) were detected. DNA analysis identified the Hb Q-Thailand mutation (α74: GAC-CAC) and the linked ($-\alpha^{4.2}$) in all cases. Analysis of α-globin gene haplotype provided the first evidence of a single origin of this Hb variant in Thai population.

Introduction

Thalassemia and hemoglobinopathies are very common in Thailand and other Asian countries. In addition to the two most common hemoglobin (Hb) variants, Hb E [β26(B8): Glu-Lys] and Hb Constant Spring (αTerm: TAA-CAA), other abnormal Hbs caused by both α-chain and β-chain variants have been reported [1]. Among those variants, Hb Q-Thailand, [α74(EF3) Asp-His] caused by a point mutation at codon 74 (GAC-CAC) of the α1-globin gene on chromosome 16p with a leftward single α-globin gene deletion ($-\alpha^{4.2}$), has occasionally been documented, mostly in the heterozygous state or in association with α^0-thalassemia. Heterozygous Hb Q-Thailand usually shows slight red blood cell microcytosis because of the linked ($-\alpha^{4.2}$) α^+-thalassemia allele. Co-inheritance with α^0-thalassemia leads to a clinical phenotype of the Hb Q-H disease with clinical features similar to the deletional Hb H disease [2–5]. In contrast, interactions of Hb Q-Thailand with other hemoglobinopathies are relatively rare. Association of Hb Q-Thailand with Hb E has been described only in Singaporean and Thai families [6,7], whereas the Hb QE/Bart's disease has been documented in a Chinese patient with the thalassemia intermedia phenotype [8]. Interaction of the α^{QT}-Thailand and the β^E-globin chains in the patients could lead to the formation of the Hb QE with different analytical characteristics with that of Hb Q-Thailand. Therefore, association of the Hb Q-Thailand with other globin gene disorders has important implications in clinical manifestation, laboratory diagnosis and genetic counseling. In addition, although Hb Q-Thailand has been found in various populations, data on its origin and spread remains to be elucidated.

In this paper we described molecular characteristics and hematological profiles of syndromes caused by interactions of Hb Q-Thailand with several hemoglobinopathies in 52 unrelated Thai patients, including as many as 10 different genotypes, the largest series reported to date. Analytical characteristics on both HPLC and capillary electrophoresis, molecular and hematological features, as well as α-globin gene haplotype associated with this Hb variant are presented.

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Materials and methods

Subjects and hematological analysis

Ethical approval of the study protocol was obtained from our Institutional Review Board (IRB) at Khon Kaen University (HE481115) and informed consent was obtained. Blood anticoagulated with EDTA was obtained from 52 unrelated adult Thai individuals with Hb Q-Thailand who were selectively recruited from our ongoing thalassemia screening program at the Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand. Hematological parameters were obtained using a standard automated blood cell counter (Coulter Gen S; Coulter Electronics, Hialeah, FL, USA). Hb analysis was performed using automated Hb-HPLC analyzer (Variant™; Bio-Rad Laboratories, Hercules, CA, USA) and automated capillary zone electrophoresis (Capillarys 2: Sebia, Lisses, France) [9].

DNA and α-globin gene haplotype analyses

Genomic DNA was prepared from blood leukocytes of the patients using the standard method. Detection of Hb Q-Thailand mutation and the linked 4.2 kb deletion α^+-thalassemia was carried out using a multiplex allele-specific PCR as described previously [7]. Identifications of the α^0-thalassemia (SEA and THAI deletions), α^+-thalassemia (3.7 and 4.2 kb deletions), Hb Constant Spring and Hb Paksé were routinely performed in our laboratory using PCR methods described elsewhere [10–12]. The β^E and β-thalassemia mutations were identified using allele-specific PCR assays [13,14]. Analysis of α-globin gene haplotype including 7 polymorphic sites, 5′ζ2 *Xba* I, interζ *Bgl* I, interζ HVR triallelic region, ψα1 *Acc* I, α2 *Rsa* I, α1 *Pst* I and θ1 *Pst* I sites, was carried out using PCR and restriction digestions as described [15].

Results

We divided 52 Thai subjects with Hb Q-Thailand into 4 groups according to genotypes. DNA analysis by multiplex PCR identified the Hb Q-Thailand mutation (α74: GAC-CAC) and the linked 4.2 kb deletion α^+-thalassemia ($-\alpha^{4.2}$) in all cases (Fig. 1). Twenty six subjects with heterozygous ($-\alpha^{QT}/\alpha\alpha$) and a homozygous ($-\alpha^{QT}/-\alpha^{QT}$) Hb Q-Thailand were presented in group I where as those with interactions with other thalassemia genes including α^+-thalassemia ($-\alpha^{3.7}$), α^0-thalassemia ($-^{SEA}$), Hb Constant Spring ($\alpha^{CS}\alpha$), Hb E (β^E) and β^0-thalassemia (β^0) were categorized into groups II–IV. Hematological phenotypes of these subjects are presented in Table 1. As shown in the table, heterozygous and homozygous Hb Q-Thailand (Group I) had very mild hypochromic microcytic anemia. The levels of Hb F were within the normal range in all cases. In heterozygotes, the level of Hb Q-Thailand ($\alpha^{QT}{}_2\beta^A{}_2$) was at 29.8 ± 8.0% and Hb A_2 ($\alpha^A{}_2\delta_2$) was within normal range (2.6 ± 1.0%). In contrast, a major peak of Hb Q-Thailand (82.3%) was observed in a homozygous subject and no Hb A_2 was detected as the patient had no α^A globin chain. We observed no Hb H (β_4) and Hb Bart's (γ_4) in this case. It was found that on HPLC analysis, Hb Q-Thailand and its derivative, the Hb QA_2 ($\alpha^{QT}{}_2\delta_2$), were not distinctly separated (Fig. 2A) but they were clearly identified on the capillary electrophoresis system (Fig. 2D).

Among the 9 subjects in group II with interactions of Hb Q-Thailand and other α-thalassemias, the most common was the interaction with α^0-thalassemia (SEA deletion), causing the Hb Q-H disease ($-\alpha^{QT}/-^{SEA}$), which was found in 6 subjects. The patients had mild to moderate hypochromic microcytic anemia, characteristics of Hb H disease. Hb analysis showed a major peak of Hb Q-Thailand (79.4 ± 8.3%) in addition to Hb H and Bart's. As shown in Table 1, this Hb Q-H disease appeared to have a similar hematological phenotype with that of the deletional Hb H disease commonly encountered in our routine practice [11]. Of the 3 remaining cases in this group, two were double heterozygotes for Hb Q-Thailand and Hb Constant Spring ($-\alpha^{QT}/\alpha^{CS}\alpha$) and 1 was a double heterozygote for Hb Q-Thailand and α^+-thalassemia ($-\alpha^{QT}/-\alpha^{3.7}$). All of them had mild hypochromic microcytic anemia but no Hb H and Hb Bart's was detected. The levels of Hb Q-Thailand were found to be 49.2% and 49.3% in the former and 34.7% in the latter genotypes, respectively.

Group III included those with Hb Q-Thailand found in association with Hb E in various combinations including 7 double heterozygotes for Hb Q-Thailand/Hb E ($-\alpha^{QT}/\alpha\alpha$, β^E/β^A), 3 double Hb Q-Thailand/Hb E and α^+-thalassemia ($-\alpha^{QT}/-\alpha^{3.7}$, β^E/β^A), 3 homozygous Hb E/Hb Q-Thailand ($-\alpha^{QT}/\alpha\alpha$, β^E/β^E) and a homozygous Hb E with compound Hb Q-Thailand/Hb Constant Spring ($-\alpha^{QT}/\alpha^{CS}\alpha$, β^E/β^E). In this group, in addition to Hb E ($\alpha^A{}_2\beta^E{}_2$) and Hb Q-Thailand ($\alpha^{QT}{}_2\beta^A{}_2$) fractions, a small peak of the Hb QE resulted from the ($\alpha^{QT}{}_2\beta^E{}_2$) tetrameric assembly with slower separation times was observed on both HPLC and capillary electrophoresis (Fig. 2B and E). However, as shown in the figures, capillary electrophoresis provided better separation of the Hb Q-Thailand and its Hb QE derivative. As expected, this Hb QE rather than Hb Q-Thailand was detected in all 3 homozygous Hb E/Hb Q-Thailand and a homozygous Hb E with compound Hb Q-Thailand/Hb Constant Spring since all these patients had no β^A-globin chain, required for the formation of Hb Q-Thailand.

The remaining two cases in group IV were found to be double heterozygotes for Hb Q-Thailand and β^0-thalassemia. DNA analysis of β-globin gene identified the 4 bp deletions between codons 41/42, in both cases, the most common β-thalassemia mutation in our region [16]. In these cases, Hb analysis demonstrated, in addition to Hb Q-Thailand and elevated Hb A_2 ($\alpha^A{}_2\delta_2$), 1.0% and 2.6% of the Hb QA_2 derivative ($\alpha^{QT}{}_2\delta_2$) was observed (Fig. 2C). It is noteworthy that diagnosis of β-thalassemia in these two cases was not altered due to the co-inheritance of Hb Q-Thailand, as Hb A_2 levels (4.9% and 5.3%)

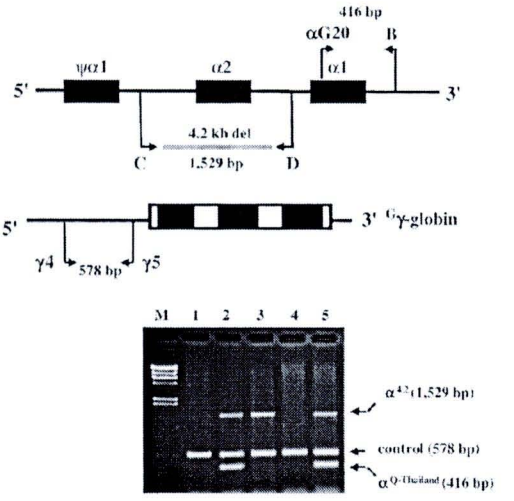

Fig. 1. A multiplex allele-specific PCR assay for simultaneous detection of the $-\alpha^{4.2}$ and the *in cis* $\alpha^{Q\text{-Thailand}}$ mutation. The locations and orientations of primers (C and D) and (αG20 and B) for detection of the $-\alpha^{4.2}$ (1529 bp) and $\alpha^{Q\text{-Thailand}}$ (416 bp) mutations are indicated. The 578 bp is an internal control fragment of the $^G\gamma$-globin gene promoter. M: λ/*Hind* III size markers. 1 and 4: negative for Hb Q-Thailand, 2 and 5: positive for Hb Q-Thailand and 3: α^+-thalassemia carrier with 4.2 kb deletion ($-\alpha^{4.2}/\alpha\alpha$).

Table 1
Hematological parameters and globin genotypes of the Hb Q-Thailand related disorders. Percentage of Hbs A_2/E, F, QT and QE were based on HPLC analyzer. Hb QA_2 was recorded on the capillary electrophoresis. Values are presented as mean ± SD, range or as raw data where appropriate.

Gr.	Genotype (n)	Rbc (x 10^{12}/l)	Hb (g/dl)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	Hb A_2/E (%)	Hb QT (%)	Hb QE (%)	Hb QA_2 (%)
I	$-\alpha^{QT}/\alpha\alpha$ (26)	5.2 ± 0.6	12.8 ± 1.7	38.2 ± 5.0	74.9 ± 6.9	24.8 ± 0.6	33.6 ± 1.6	14.4 ± 2.4	2.6 ± 1.0	29.8 ± 8.0	None	None
	$-\alpha^{QT}/-\alpha^{QT}$ (1)	5.5	12.8	40.0	72.2	23.1	32.0	16.4	None	82.3	None	None
II	$-\alpha^{QT}/-\alpha^{3.7}$ (1)	4.5	9.7	31.0	70.0	22.2	31.0	15.7	None	34.7	None	None
	$-\alpha^{QT}/\alpha^{CS}\alpha$ (2)	4.3, 4.6	9.7, 9.9	29.5, 31.6	69.1, 69.5	21.4, 23.2	30.7, 33.6	16.3, 15.9	1.5, 2.0	49.2, 49.3	None	None
	$-\alpha^{QT}/--^{FSA}$ (6)	4.4 ± 0.5	8.0 ± 1.2	28.2 ± 2.3	70.2 ± 3.6	19.7 ± 4.5	29.4 ± 1.6	23.8 ± 1.8	None	79.4 ± 8.3	None	< 1.0
III	$-\alpha^{QT}/\alpha\alpha$ (7) β^E/β^A	5.3 ± 0.4	12.6 ± 2.3	38.2 ± 7.5	78.3 ± 4.7	25.8 ± 1.4	33.2 ± 1.0	14.4 ± 1.4	19.7 ± 1.6	19.1 ± 4.3	6.7 ± 1.8	None
	$-\alpha^{QT}/-\alpha^{3.7}$ (3) β^E/β^A	4.9–6.7	13.3–15.6	37.5–46.6	62.0–69.0	22.3–23.1	33.4–36.0	12.3–15.5	9.1–15.6	29.8–33.7	8.0–11.1	None
	$-\alpha^{QT}/\alpha\alpha$ (3) β^E/β^E	5.8 ± 0.9	12.5 ± 1.5	37.9 ± 4.6	66.1 ± 3.0	21.7 ± 0.7	32.9 ± 0.5	15.9 ± 0.6	75.4 ± 4.3	None	14.4 ± 0.7	None
	$-\alpha^{QT}/\alpha^{CS}\alpha$ (1) β^E/β^E	5.5	11.7	37.6	68.2	21.2	31.5	15.2	44.8	None	50.1	None
IV	$-\alpha^{QT}/\alpha\alpha$ (2) β^0/β^A	4.8, 4.6	10.0, 11.0	31.3, 31.6	65.3, 68.0	20.8, 23.2	31.9, 32.0	15.6, 15.3	4.9, 5.3	13.8, 16.6	None	1.0, 2.6

were still higher than normal. Other hematological parameters were as usually observed for a β-thalassemia carrier.

Table 2 demonstrated the α-globin gene haplotypes associated with normal α-globin gene, Hb Q-Thailand and the $(-\alpha^{4.2})$ α-thalassemia determinants in Thai population. Among 52 subjects studied, complete segregation could be obtained from 14 Hb Q-Thailand alleles. All of them are associated with a single haplotype; (+ − S + 0 − −). As shown in the table, this haplotype is exactly the same with that of the 4 $(-\alpha^{4.2})$ α-thalassemia determinant and is one of the common haplotypes observed for normal α-globin genes in Thai population.

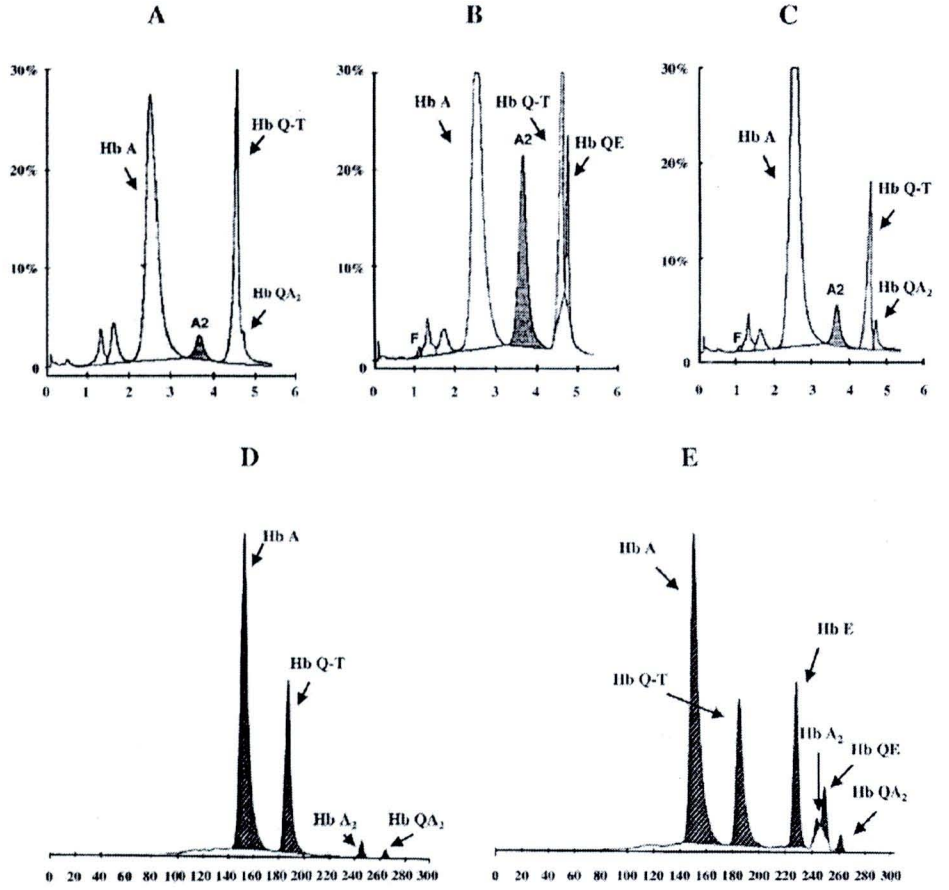

Fig. 2. Representative Hb analysis demonstrating Hb Q-Thailand variant using automated HPLC (A, B and C) and capillary electrophoresis (D and E). A and D, Hb Q-Thailand heterozygote. B and E, double heterozygote for Hb Q-Thailand and Hb E. C, double Hb Q-Thailand/β^0-thalassemia. Hb A, Hb Q-Thailand, Hb QE and Hb QA_2 are indicated by arrows.

Discussion

Hb Q-Thailand is among rare examples of α-globin chain structural variant alleles that are localized to the chromosome that also contains an α-thalassemia determinant. It has only been reported in China and Southeast Asian countries, mostly as heterozygotes or compound heterozygotes with α^0-thalassemia, causing Hb Q-H disease [2–5]. Interactions with other hemoglobinopathies have been rarely documented in this region [6–8]. We have studied 52 unrelated Thai subjects with this Hb variant, the largest series described to date of this relatively uncommon but clinically important disorder. The heterozygous form of Hb Q-Thailand does not have clinical symptom and is associated with only slight hypochromic microcytosis. Although no homozygous form has been documented so far for comparison of the hematological phenotype, homozygous Hb Q-Thailand is also a mild condition associated with only slight hypochromic microcytosis and does not lead to clinical signs of Hb H disease. Most of the Hb observed in this case was Hb Q-Thailand (82.3%) (Table 1). This finding likely indicates that despite a structural change of the Hb molecule, Hb Q-Thailand has similar functional properties to that of normal Hb A. It is noteworthy as for other α-globin chain variants that at least two Hb derivatives should be observed for individuals with this Hb variant i.e. the Hb Q-Thailand ($\alpha^{QT}{}_2\beta^A{}_2$) and the Hb QA_2 ($\alpha^{QT}{}_2\delta_2$) derivative. Both HPLC and capillary electrophoresis systems could demonstrate these although we noted that the latter provides better separation of these two Hb molecules (Fig. 2A and D).

Double heterozygote for Hb Q-Thailand and α^+-thalassemia ($-\alpha^{QT}/-\alpha^{3.7}$) or Hb Constant Spring ($-\alpha^{QT}/\alpha^{CS}\alpha$) had similar phenotypic features with that of the homozygous Hb Q-Thailand or homozygous α^+-thalassemia, although with apparently more anemia. As for the homozygous form, these combinations do not lead to the Hb H disease. The affected individuals are clinically normal, have minimal anemia and reduced MCV and MCH. The levels of Hb Q-Thailand for the ($-\alpha^{QT}/-\alpha^{CS}\alpha$) genotype was much higher than that of the ($-\alpha^{QT}/-\alpha^{3.7}$) genotype (49.2% and 49.3% versus 34.7%), the data indicating a lower proportion of Hb A for the former genotype. Hb Constant Spring is an elongated and unstable α-globin variant resulted from a termination codon mutation of an α2-globin gene [17]. This $\alpha^{\text{Constant Spring}}$ mutation results in loss of approximately 98% of expression from the mutated α2-globin gene [18]. Hb A is synthesized alternatively from α1-globin gene. There are three α-globin gene defects including α^{QT}, $-\alpha^{4.2}$ and $\alpha^{\text{Constant Spring}}$ for the ($-\alpha^{QT}/\alpha^{CS}\alpha$) genotype and α^{QT}, $-\alpha^{4.2}$ and $-\alpha^{3.7}$ for the ($-\alpha^{QT}/-\alpha^{3.7}$) genotype. Only one α-globin gene remains i.e. the hybrid α2α1-globin gene (due to the 3.7 kb deletion) in the ($-\alpha^{QT}/-\alpha^{3.7}$) genotype and an intact α1-globin gene in the ($-\alpha^{QT}/\alpha^{CS}\alpha$) genotype. Our finding indirectly confirms that much Hb A is produced from the hybrid α2α1-globin gene as compared to the intact α1-globin gene.

In contrast, association of the Hb Q-Thailand with α^0-thalassemia ($-\alpha^{QT}/-^{SEA}$) in 6 Thai subjects in group II resulted in the clinically important Hb Q-H disease with thalassemia intermedia phenotype. The hematological findings of these 6 cases were indistinguishable from those of the classical deletional Hb H disease in our records [11], except that the major Hb molecule detected in the 6 cases was the Hb Q-Thailand (79.4 ± 8.3%) instead of Hb A, the remaining being Hb Bart's and Hb H. Interaction of Hb Q-Thailand with α^0-thalassemia leads to three α globin gene deletion, so that only α^{QT}-globin chain is synthesized. Since we observed no Hb A_2 and minimal levels Hb QA_2 in these cases, it is likely that most of the α^{QT}-globin chain forms tetramer with β^A-globin chain ($\alpha^{QT}{}_2\beta^A{}_2$) resulting in Hb Q-Thailand. Accordingly, in Hb Q-H disease, we could observe Hb Q-Thailand, Hb Bart's and Hb H but Hb A is absent. Hb Q-H disease is thought to be rare in Southeast Asian populations and all cases reported so far have been Chinese or of Chinese origin [3–6,19]. Identification of 6 Thai patients in this study indicates that the disease may not be uncommon among Southeast Asian population.

Association of Hb Q-Thailand with heterozygous Hb E has been reported sporadically in Thai, Chinese and Singaporean [6,7,20]. However, as shown in Table 1 group III, we found as many as 4 genotypes among 14 Thai subjects with this interaction. Two of them, the Hb Q-Thailand/α^+-thalassemia/Hb E heterozygote ($-\alpha^{QT}/-\alpha^{3.7}$, β^E/β^A) and the compound heterozygous Hb Q-Thailand/Hb Constant Spring/homozygous Hb E ($-\alpha^{QT}/\alpha^{CS}\alpha,\beta^E/\beta^E$) have not been described before. As shown in Table 1, we observed similar phenotypic features of these two novel genotypes with the 3 cases of Hb Q-Thailand trait/homozygous Hb E ($-\alpha^{QT}/\alpha\alpha,\beta^E/\beta^E$) reported in this study and that of a pregnant woman with the same genotype reported previously [7]. These complex interactions between Hb Q-Thailand, Hb Constant Spring, α^+-thalassemia and Hb E are associated with mild clinical phenotypes and do not lead to complex α/β-thalassemia syndromes known as the AEBart's, EFBart's and Constant Spring EEBart's diseases occasionally encountered among Thai population [11,21,22]. This could be best explained by the fact that compound heterozygote for Hb Q-Thailand and α^+-thalassemia ($-\alpha^{QT}/-\alpha^{3.7}$) or Hb Constant Spring ($-\alpha^{QT}/\alpha^{CS}\alpha$) does not result in the Hb H disease. However, with these combinations, in addition to Hb E, at least 3 other Hb variants would be expected i.e. Hb Q-Thailand and Hb QA_2 mentioned above and Hb QE resulted from the ($\alpha^{QT}{}_2\beta^E{}_2$) tetrameric assembly. Again, HPLC analysis could demonstrate only Hb Q-Thailand and Hb QE (Fig. 2B) whereas all the three variants could be clearly observed on capillary electrophoresis (Fig. 2E). However, only Hb E and Hb QE were detected in those with homozygous Hb E. The lower proportions of Hb E in heterozygote with these complex interactions are not unexpected. We have demonstrated previously the lower proportions of Hb E in Hb E heterozygotes with various forms of α-thalassemia [10].

In the last two subjects with double heterozygote for Hb Q-Thailand and β^0-thalassemia, we found similar hematological parameters with those usually observed for pure β-thalassemia carriers with mild hypochromic microcytic red cell and slightly reduced hemoglobin values. Because of the elevated Hb A_2, Hb analysis could clearly demonstrate both Hb Q-Thailand and Hb QA_2 (Fig. 2C). It has been

Table 2
α-Globin gene haplotypes associated with $\alpha^{\text{Q-Thailand}}$, α^+-thalassemia (4.2 kb deletion) and normal α-globin genes in Thai population. Numbers indicate number of alleles associated with each specific haplotype. + and − indicate the presence and absence of each polymorphic site where as 0 indicates deletion. nd = not done.

Globin alleles	*Xba* I	*Bgl* I	*Sac*I	*Acc* I	*Rsa* I	α *Pst* I	θ *Pst* I	Number of allele
αα*	+	−	S	+	+	−	nd	10
	+	−	S	+	−	−	nd	21
	+	−	S	+	−	+	nd	1
	+	−	S	−	−	−	nd	1
$-\alpha^{4.2}$	+	−	S	+	0	−	−	4
$-\alpha^{\text{Q-Thailand}}$	+	−	S	+	0	−	−	14

* Data from our previous report [15].

noted previously in a Chinese patient that association of Hb Q-Thailand with β-thalassemia could result in a normal Hb A_2 value and a possible mis-diagnosis of β-thalassemia carrier [23]. This is not the case for our Thai patients, the levels of Hb A_2 were found to be 4.9% and 5.3%, which are still within the diagnostic range for a typical β-thalassemia carrier. The levels of Hb QA_2 as measured by capillary electrophoresis were 1.0% and 2.6%. In fact at routine diagnostic, one should obtain a total Hb A_2 level by combining the levels of Hb A_2 and Hb QA_2 before making a diagnosis of a β-thalassemia carrier. We conclude that although co-inheritance of α-thalassemia (including Hb Q-Thailand) with β-thalassemia carrier may lead to a reduction in the level of Hb A_2, this does not interfere with the diagnosis of the β-thalassemia carrier. The same finding has been noted previously in a double heterozygous α^0-thalassemia and β-thalassemia trait [14].

Although Hb Q-Thailand has been described in many Asian populations, the data on its origin and spread remains to be elucidated. In this study, we found that a single α-globin gene haplotype, (+ − − S + 0 − − −), was associated with all 14 Thai ($-\alpha^{Q\text{-Thailand}}$) alleles segregated. It is the same with that of the α^+-thalassemia chromosome ($-\alpha^{4.2}$) examined (Table 2). Since all Hb Q-Thailand are observed in linkage with this α^+-thalassemia deletion and no Hb Q-Thailand has been identified in individuals with a chromosome containing two intact α-globin genes, it is most likely that these linked abnormalities arose by point mutation in already existing ($-\alpha^{4.2}$) chromosome. Although there are no haplotype studies on the $\alpha^{Q\text{-Thailand}}$ chromosomes in other populations, our data indicates a unique evolutionary origin of the $\alpha^{Q\text{-Thailand}}$ mutation in the Thai population. Further investigation on other populations especially in China would provide additional information related to the origin and spread of this Hb variant. Nonetheless, our result demonstrated that Hb Q-Thailand may not be uncommon as previously thought. In fact, interactions of this variant with other forms of thalassemia and hemoglobinopathies lead to complex thalassemia syndromes with various phenotypic features. Use of both hematologic and molecular analyses is essential for providing accurate diagnosis, appropriate management, and genetic counseling of the patients.

Acknowledgments

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References

[1] S. Fucharoen, P. Winichagoon, Hemoglobinopathies in Southeast Asia, Hemoglobin 11 (1987) 65–88.
[2] F.Y. Zeng, S. Fucharoen, S.Z. Huang, G.P. Rodgers, Hb Q-Thailand [α 74(EF3) Asp→His]: gene organization, molecular structure, and DNA diagnosis, Hemoglobin 16 (1992) 481–491.
[3] P. Beris, P. Huber, P.A. Miescher, J.B. Wilson, A. Kutlar, S.S. Chen, T.H. Huisman, Hb Q-Thailand-Hb H disease in a Chinese living in Geneva, Switzerland: characterization of the variant and identification of the two α-thalassemic chromosomes, Am. J. Hematol. 24 (1987) 395–400.
[4] K.F. Leung, E.S. Ma, A.Y. Chan, L.C. Chan, Clinical phenotype of haemoglobin Q-H disease, J. Clin. Pathol. 57 (2004) 81–82.
[5] D. Li, C. Liao, X. Xie, H. Zhong, J. Li, Four cases of Hb Q-H disease found in Southern China, Hemoglobin 31 (2007) 109–111.
[6] J.A.M.A. Tan, J.S.H. Tay, Y.C. Wong, S.K.Y. Kham, N.B.A. Aziz, S.H. Teo, B.B. Wong, Molecular analysis of Hb Q-H and HbQ-Hb E in a Singaporean family, Southeast Asian J. Trop. Med. Public Health 850 (1995) 415–419.
[7] K. Sanchaisuriya, S. Chunpanich, S. Fucharoen, G. Fucharoen, P. Sanchaisuriya, Y. Changtrakun, Association of Hb Q-Thailand with homozygous Hb E and heterozygous Hb Constant Spring in pregnancy, Eur. J. Haematol. 74 (2005) 221–227.
[8] W. Zheng, Y. Liu, D. Chen, K. Rong, Y. Ge, C. Gong, H. Chen, Complex interaction of Hb Q-Thailand and Hb E with α^0-thalassemia and hereditary persistence of fetal hemoglobin in a Chinese family, Ann. Hematol. 89 (9) (2010) 883–888.
[9] H. Srivorakun, G. Fucharoen, N. Sae-ung, K. Sanchaisuriya, T. Ratanasiri, S. Fucharoen, Analysis of fetal blood using capillary electrophoresis system: a simple method for prenatal diagnosis of severe thalassemia diseases, Eur. J. Haematol. 83 (2009) 57–65.
[10] K. Sanchaisuriya, G. Fucharoen, N. Sae-ung, A. Jetsrisuparb, S. Fucharoen, (2003) Molecular and hematologic features of hemoglobin E heterozygotes with different forms of α-thalassemia in Thailand, Ann. Hematol. 82 (2003) 612–616.
[11] S. Boonsa, K. Sanchaisuriya, G. Fucharoen, S. Wiangnon, A. Jetsrisuparb, S. Fucharoen, The diverse molecular basis and hematologic features of Hb H and AEBart's diseases in northeast Thailand, Acta Haematol. 111 (2004) 149–154.
[12] N. Sae-ung, G. Fucharoen, K. Sanchaisuriya, S. Fucharoen, α^0-thalassemia and related disorders in northeast Thailand: a molecular and hematological characterization, Acta Haematol. 117 (2007) 78–82.
[13] S. Fucharoen, G. Fucharoen, T. Ratanasiri, A. Jetsrisuparp, Y. Fukumaki, A simple non-radioactive assay for hemoglobin E gene in prenatal diagnosis, Clin. Chim. Acta 229 (1994) 197–203.
[14] N. Siriratmanawong, G. Fucharoen, K. Sanchaisuriya, T. Ratanasiri, S. Fucharoen, Rapid and simultaneous detection of β-thalassemia and α-thalassemia 1 (SEA type) in prenatal diagnosis of complex thalassemia syndrome, Clin. Biochem. 34 (2001) 377–380.
[15] S. Singsanan, G. Fucharoen, O. Savongsy, K. Sanchaisuriya, S. Fucharoen, Molecular characterization and origins of Hb Constant Spring and Hb Paksé in Southeast Asian populations, Ann. Hematol. 86 (2007) 665–669.
[16] L. Nuntakarn, S. Fucharoen, G. Fucharoen, K. Sanchaisuriya, A. Jetsrisuparb, S. Wiangnon, Molecular, hematological and clinical aspects of thalassemia major and thalassemia intermedia associated with Hb E-β-thalassemia in northeast Thailand, Blood Cells Mol. Dis. 42 (2009) 32–35.
[17] S.L. Schrier, A. Bunyaratvej, A. Khuhapinant, S. Fucharoen, M. Alijurt, L.M. Snyder, C.R. Keifer, L. Ma, N. Mohandas, The unusual pathobiology of hemoglobin Constant Spring red blood cells, Blood 89 (1997) 1762–1769.
[18] S.A. Liebhaber, J.E. Russell, Expression and developmental control of the human α-globin gene cluster, Ann. N.Y. Acad. Sci. 850 (1998) 54–63.
[19] M. Lin, J.R. Wu, L.Y. Yang, Hb Q-H disease: two cases in a Cantonese family, Blood Cells Mol. Dis. 41 (2008) 259–260.
[20] D. Li, C. Liao, J. Li, X. Xie, H. Zhong, Association of Hb Q-Thailand with heterozygous Hb E in a Chinese patient, Hemoglobin 32 (2008) 319–321.
[21] S. Fucharoen, P. Winichagoon, N. Sirikhanaratkul, J. Chowthaworn, P. Pootrakul, α And β thalassemia in Thailand, Ann. N.Y. Acad. Sci. 850 (1998) 412–414.
[22] S. Fucharoen, G. Fucharoen, N. Sae-ung, K. Sanchaisuriya, Thalassemia intermedia associated with the Hb Constant Spring EE Bart's disease in pregnancy: a molecular and hematological analysis, Blood Cells Mol. Dis. 39 (2007) 195–198.
[23] C. Liao, J. Li, D. Li, Association of β-thalassemia and Hb Q-Thailand resulting in a normal Hb A_2 value, Hemoglobin 32 (2008) 505–508.

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Secondary Erythrocytosis Caused by Hemoglobin Tak/$(\delta\beta)^0$-Thalassemia Syndrome

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Key Words
Hemoglobin Tak · Homozygous hemoglobin Tak · Secondary erythrocytosis · $(\delta\beta)^0$-Thalassemia

Abstract
Secondary erythrocytosis may arise from several causes, but an association with oxygen transport is rare. We describe for the first time a form of secondary erythrocytosis caused by compound heterozygosity for hemoglobin (Hb) Tak and $(\delta\beta)^0$-thalassemia found in an adult Thai individual. The patient had marked erythrocytosis and microcytosis with increased Hb and hematocrit values. Hb analyses using the Hb Gold Analyzer showed Hb A_2 (72.5%) and Hb F (30.0%) without Hb A while the capillary electrophoresis revealed 2.3% Hb A_2 and a major peak of Hb F (91.2%). Further molecular investigation identified that he was in fact a compound heterozygote for Hb Tak and deletional $(\delta\beta)^0$-thalassemia. Hematological parameters of the patient were compared with those observed for a Thai boy who demonstrated features of erythrocytosis and microcytosis caused by homozygous Hb Tak with α^+-thalassemia and with those of pure carriers of Hb Tak and $(\delta\beta)^0$-thalassemia in our series. This report confirms the importance of both Hb and molecular investigations for the assessment of genotype/phenotype correlation and the appropriate management of the patients.

Introduction

Erythrocytosis encompasses a number of disorders characterized by increased circulating red blood cells (RBCs) which can be classified into primary, secondary and relative erythrocytosis. Secondary erythrocytosis may arise from several causes including inappropriate erythropoietin production, renal tumors and other kidney diseases, but association with defective oxygen transport is rare and usually caused by abnormal hemoglobins (Hbs) with increased oxygen affinity. Most patients with this condition were in essentially good health but had higher than normal RBC and Hb levels in the blood [1].

Hb Tak [β147 Term→Thr] is an abnormal Hb caused by the insertion of dinucleotide AC after codon 146, a termination codon of the β-globin gene, leading to a synthesis of the abnormal β-globin chain with an extended 11-amino-acid residue. It is one of the most commonly encountered Hb variants in Thailand and other Southeast Asian countries [2–4]. In addition to a β-thalassemic-like defect, Hb Tak has an increased oxygen affinity [5]. Although clinically asymptomatic in heterozygous form, homozygote or inheritance together with other he-

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moglobinopathies can result in significant clinical conditions including secondary erythrocytosis [6–8]. δβ-Thalassemia is a heterogeneous disorder characterized by increased production of Hb F in adult life. In heterozygotes, Hb F levels usually range from 5 to 15%, and RBC indices are reduced. Individuals with this disorder exhibit mild clinical symptoms compared with those with typical β-thalassemia, due to the beneficial effect of Hb F on RBC production and survival [9]. In Thailand, it is the most common form of high Hb F found in the population which results from a 12.5-kb DNA deletion removing β- and δ-globin genes. Interactions of $(\delta\beta)^0$-thalassemia with other thalassemias and hemoglobinopathies occasionally found in the Thai population are usually associated with thalassemia intermedia phenotypes [10, 11].

We report an adult Thai patient with a phenotype of secondary erythrocytosis and microcytosis caused by a hitherto undescribed coinheritance of Hb Tak and $(\delta\beta)^0$-thalassemia. Hematological data of the patient were compared with those of a Thai boy with features of erythrocytosis caused by homozygous Hb Tak and α^+-thalassemia and with those of Hb Tak carriers and $(\delta\beta)^0$-thalassemia carriers in our series.

Materials and Methods

Subjects

The patient was a 48-year-old man who was essentially in good health but was plethoric in appearance and had markedly elevated RBC and Hb levels. Since initial Hb analysis revealed an unknown abnormal Hb with an elevated Hb F level, a blood specimen was sent to Khon Kaen University for further analysis. The second case was a 12-year-old Thai boy with a similar erythrocytosis phenotype encountered at our center. Physical examination revealed unremarkable change but plethora and recurrent headaches. Routine complete blood count showed an elevated RBC count and Hb level. Hb analysis revealed an unknown Hb variant without Hb A. Therefore, molecular investigation was performed. Additional subjects with Hb Tak and $(\delta\beta)^0$-thalassemia were from our earlier reports [11, 12] and selectively recruited from our ongoing thalassemia screening program at our center. Ethical approval of the study protocol was obtained from the institutional review board of Khon Kaen University (HE481115).

Hematological and DNA Analyses

Hematological parameters were obtained using a standard automated blood cell counter (Coulter T series; Beckman-Coulter Co., Hialeah, Fla., USA). Hb analysis was performed using an automated Hb-low pressure liquid chromatography (LPLC) analyzer (Hb Gold; Drew Scientific, Ltd., Barrow-in-Furness, UK) and automated capillary zone electrophoresis (Capillarys 2; Sebia, Lisses, France) [13]. Identifications of the α^0-thalassemia (SEA and THAI deletions), α^+-thalassemia (3.7- and 4.2-kb deletions), Hb Constant Spring and Hb Paksé mutations were routinely performed in our laboratory using PCR methods described elsewhere [14, 15]. Identification of Hb Tak mutation and screening for common high Hb F determinants in Thailand including $(\delta\beta)^0$-thalassemia, $^G\gamma(^A\gamma\delta\beta)^0$-thalassemia and hereditary persistence of fetal Hb were performed using multiplex PCR assays as described previously [11, 12]. Confirmation of the homozygosity for Hb Tak was done by family analysis and direct DNA sequencing.

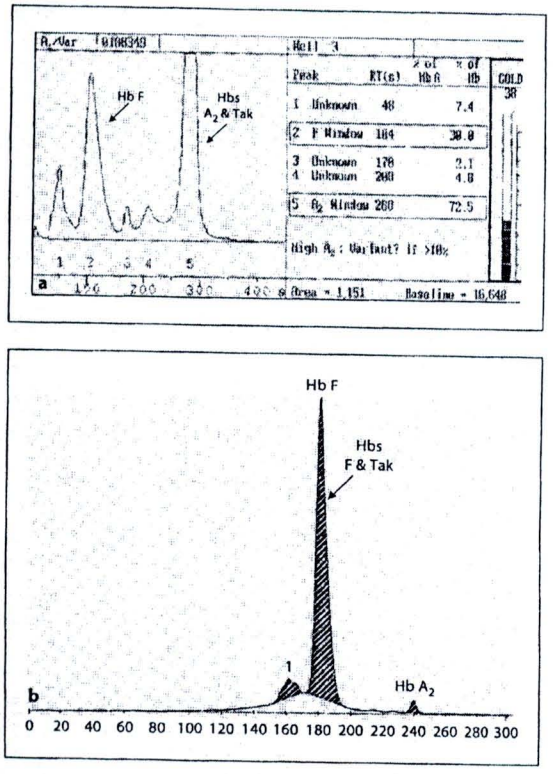
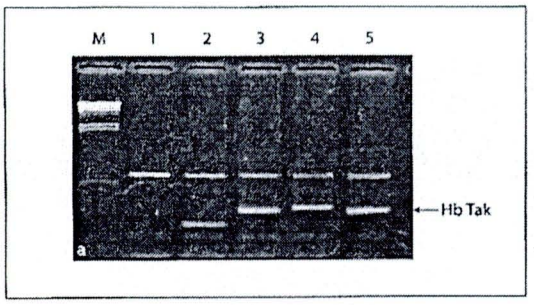


Fig. 1. Hb analysis of the proband demonstrating the Hb Tak variant coeluted with Hb A_2 at the A_2 window on the automated LPLC analyzer (**a**) but comigrating with Hb F on the capillary electrophoresis system (**b**).

Result

The patient had no family history of blood disorder. He was essentially in good health but was plethoric in appearance. As shown in table 1, he had marked erythrocytosis and microcytosis with RBCs 8.7 × 10^{12}/l, Hb 19.5 g/dl, hematocrit (Hct) 59.2%, mean corpuscular volume



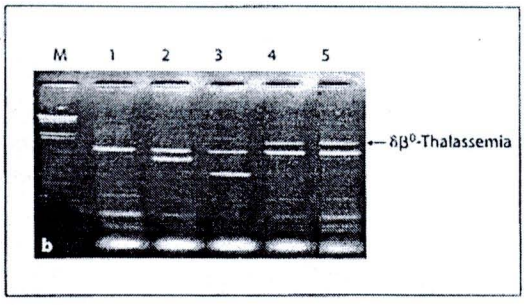


Fig. 2. a Agarose gel electrophoresis of the multiplex allele-specific PCR analysis for identification of Hb Tak, Hb S and Hb D-Punjab mutations. Lane 1 = Normal control; lane 2 = Hb S carrier; lane 3 = Hb Tak carrier; lane 4 = Hb D-Punjab carrier; lane 5 = the proband. M represents the λ/*Hin*dIII size markers. **b** Multiplex PCR analysis for the detection of common high Hb F determinants in Thailand. Lane 1 = Normal control; lane 2 = HPFH-6 carrier; lane 3 = deletion-inversion $^{G}\gamma(^{A}\gamma\delta\beta)^{0}$-thalassemia carrier; lane 4 = $(\delta\beta)^{0}$-thalassemia carrier; lane 5 = the proband. M represents the λ/*Hin*dIII size markers.

Table 1. Hematological data of the proband with compound Hb Tak/$(\delta\beta)^{0}$-thalassemia syndrome compared with those of a homozygous Hb Tak with heterozygous α^{+}-thalassemia, 7 carriers of Hb Tak and 142 carriers of $(\delta\beta)^{0}$-thalassemia in our series

Parameter	Proband (HbTak/ $\delta\beta^{0}$-thalassemia)	Homozygous Hb Tak with α^{+}-thalassemia	Hb Tak carrier	$\delta\beta^{0}$-Thalassemia carrier
Patients	1	1	7	142
α genotype	αα/αα	$-\alpha^{3.7}/\alpha\alpha$	αα/αα	αα/αα
β genotype	$\beta^{Tak}/(\delta\beta)^{0}$-thalassemia	β^{Tak}/β^{Tak}	β^{Tak}/β^{A}	$\beta^{A}/(\delta\beta)^{0}$-thalassemia
RBCs, $\times 10^{12}$/l	8.7	8.6	5.5 ± 0.3	4.9 ± 0.7
Hb, g/dl	19.5	18.9	14.5 ± 1.9	12.3 ± 7.6
Hct, %	59.2	60.4	42.3 ± 5.9	35.7 ± 4.7
MCV, fl	68.0	69.5	82.4 ± 3.5	75.1 ± 7.8
MCH, pg	22.4	21.8	30.4 ± 3.5	24.6 ± 2.2
MCHC, g/dl	32.9	31.3	34.8 ± 1.5	32.3 ± 1.0
Hb type	A_2, Tak, F	A_2, Tak	A_2, Tak, A	A_2, F, A
Hb A_2, %	2.3	5.4	3.5 ± 0.8	2.2 ± 0.4
Hb F, %	30.0	1.2	<1.0	20.7 ± 5.6
Hb Tak, %	61.2	91.9	28.9 ± 4.8	–

Data are presented as the mean ± SD or as raw data where appropriate.
MCHC = Mean corpuscular Hb concentration.

(MCV) 68.0 fl, mean corpuscular Hb (MCH) 22.4 pg and mean corpuscular Hb concentration 32.9 g/dl. Hb analyses using the LPLC Hb Analyzer (Hb Gold) showed Hb A_2 (72.5%) and Hb F (30.0%) without Hb A, whereas capillary electrophoresis revealed 2.3% Hb A_2, a major peak of Hb F (91.2%) but no Hb A (fig. 1). These data indicated that he carried a β-globin chain variant that was coeluted with Hb A_2 on liquid chromatography but was comigrating with Hb F on capillary electrophoresis and another high Hb F determinant. DNA analysis using multiplex PCR for identification of Hb S, D-Punjab and Hb Tak and multiplex PCR for identifying common high Hb F determinants in Thailand identified the Hb Tak mutation *in trans* to the 12.5-kb deletional $(\delta\beta)^{0}$-thalassemia determinant (fig. 2). No common α-thalassemia including α^{0}-thalassemia (SEA and THAI types), α^{+}-thalassemia

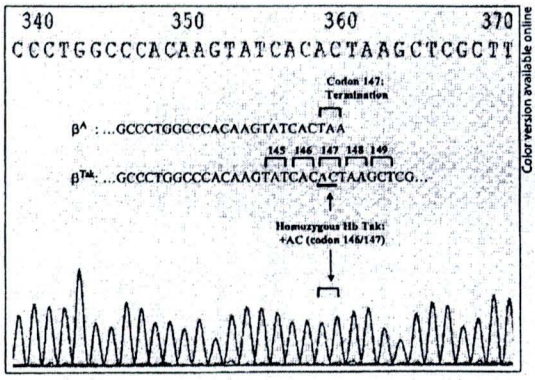


Fig. 3. Direct DNA sequencing of β-globin gene demonstrating the homozygosity for an AC insertion at the termination codon identified in the second patient with homozygous Hb Tak and α^+-thalassemia.

(3.7- and 4.2-kb deletions), $\alpha^{\text{Constant Spring}}$ and $\alpha^{\text{Paksé}}$ was detected. Therefore, he was a compound heterozygote for Hb Tak/$(\delta\beta)^0$-thalassemia, a hitherto undescribed condition.

The hematological data of the patient were compared with those observed in a Thai boy with a homozygous Hb Tak with α^+-thalassemia (3.7 kb), another undescribed condition encountered at our routine setting, and with those of 7 pure carriers of Hb Tak and 142 carriers of $(\delta\beta)^0$-thalassemia in our series (table 1). We observed a similar phenotype for this boy with homozygous Hb Tak and α^+-thalassemia. He had marked erythrocytosis and microcytosis with RBCs 8.6 × 10^{12}/l, Hb 18.9 g/dl, Hct 60.4 %, MCV 69.5 fl, MCH 21.8 pg and a mean corpuscular Hb concentration 31.3 g/dl. Hb analyses demonstrated a major peak of Hb Tak (91.9 %) and increased Hb A_2 (5.4%) but no Hb A. Screening for β-thalassemia mutations commonly found in our region [16, 17] yielded a negative result. DNA analysis by allele-specific PCR revealed homozygosity for the Hb Tak mutation and a coinheritance of the 3.7-kb deletional α^+-thalassemia determinant. As expected, further direct DNA sequencing identified homozygosity for AC insertion at the termination codon of the β-globin gene (fig. 3). In addition, family analysis revealed that his father was a compound Hb Tak/Hb E whereas his mother was a double heterozygote for Hb Tak and α^+-thalassemia (3.7-kb deletion; data not shown). Therefore, the patient obtained Hb Tak from his father and inherited both Hb Tak and α^+-thalassemia from his mother. Although plethoric in appearance and with recurrent mild headaches, he was otherwise well and had normal growth and development. In contrast, all 7 carriers of Hb Tak had RBC and Hb levels within normal ranges. Hb A_2 was at borderline level (3.5 ± 0.8%) and no microcytosis was observed. The percentage of Hb Tak was 28.9 ± 4.8%. Carriers of $(\delta\beta)^0$-thalassemia were associated with hematologically mild phenotypes. All of them had high Hb F (20.7 ± 5.6%) and normal Hb A_2 (2.2 ± 0.4%) levels. Reduced MCV (75.1 ± 7.8 fl) and MCH (24.6 ± 2.2 pg) values were noted, but RBC and Hb levels were within normal ranges.

Discussion

Secondary erythrocytosis associated with thalassemia and hemoglobinopathies is rare, and among those which produce erythrocytosis, only moderate degrees of elevation of Hb and RBC counts have generally been found. We described 2 forms of this condition caused by interactions of a high oxygen affinity Hb (the Hb Tak) with other thalassemias in Thai patients. In the first case, secondary erythrocytosis was associated with a compound heterozygosity for Hb Tak and $(\delta\beta)^0$-thalassemia. In the second case, this was associated with a homozygous Hb Tak/α^+-thalassemia. In both cases, the diseases were associated with increased RBC mass as reflected by increases in Hb and Hct values. Compound heterozygotes for Hb Tak/Hb E and Hb Tak/β^0-thalassemia and homozygous Hb Tak have mild erythrocytosis [6–8, 18]. In these cases, the Hb F levels are in the normal range or marginally elevated (usually <5%) in compensatory response to β-thalassemia alleles. Our 2 cases had secondary erythrocytosis combined with reduced RBC indices. As we observed normal MCV and MCH values in all cases of Hb Tak carriers and reduced MCV and MCH values in $(\delta\beta)^0$-thalassemia carriers (table 1), the reduced MCV and MCH observed in the 2 patients could likely be attributed to the thalassemia alleles. The raised level of Hb A_2 (5.4%) in the Hb Tak homozygote with α^+-thalassemia (3.7-kb deletion) in this report is comparable with a β-thalassemia-like condition. From the hematological data observed for a compound Hb Tak/$(\delta\beta)^0$-thalassemia syndrome with as high as 30.0% Hb F due to the $(\delta\beta)^0$-thalassemia allele in addition to a high oxygen affinity Hb Tak (61.2%), it is conceivable that in this case, erythrocytosis might be more pronounced than usual, as Hb F itself has increased oxygen affinity. Although at the time of in-

vestigation, this patient was healthy and had no experienced symptoms of headache, dizziness and chest pain that may result from hyperviscosity of the blood due to very high RBC numbers, a long-term careful follow-up and management should be taken into consideration. Similar phenotypes have been documented for cases with Hb Crete [β129 (H7) Ala→Pro], another Hb variant with high oxygen affinity found in Greek descent individuals [19].

In this study, identification of Thai patients with secondary erythrocytosis caused by interaction of thalassemia and hemoglobinopathies confirms that for areas where these genetic disorders are prevalent, atypical cases in addition to the thalassemia syndromes may result from the interaction of several defects with a spectrum of clinical and hematological manifestations. Diagnosis of these diseases may be problematic unless DNA analysis is performed. As exemplified in figure 1 for the diagnosis of a compound Hb Tak/$(\delta\beta)^0$-thalassemia, Hb Tak, Hb A_2 and Hb E are not distinctly separated on the LPLC Hb Analyzer which could lead to a misdiagnosis of Hb E/β-thalassemia. On capillary electrophoresis, it comigrates with Hb F which could alternatively lead to a misdiagnosis of β-thalassemia major. Accurate diagnosis of these clinically relevant hemoglobinopathies using both hematological and DNA analyses provides information necessary for proper clinical management and genetic counseling of the cases.

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References

1 Pearson TC: Diagnosis and classification of erythrocytoses and thrombocytoses. Baillieres Clin Haematol 1998;11:695–720.

2 Flatz G, Kinderlerer J, Kilmartin J: Hemoglobin Tak: a variant with additional residues at the end of the beta chains. Lancet 1971;i:732–733.

3 Lie-Injo LE, Randhawa ZI, Ganesan J, Kane J, Peterson D: Hemoglobin Tak in a newborn Malay. Hemoglobin 1977;1:747–757.

4 Fucharoen S, Fucharoen G, Sae-ung N, Sanchaisuriya K, Fukumaki Y: Molecular and hematological characterization of Hb Tak and H Pyrgos in Thailand. Southeast Asian J Trop Med Public Health 1997;28(suppl 3):110–114.

5 Imai K, Tientadakul O, Opartkiattikul O: Detection of haemoglobin variants and inference of their functional properties using complete oxygen dissociation curve measurements. Br J Haematol 2001;112:483–487.

6 Hoyer JD, Wick M, Thibodeau S: Hb Tak confirmed by DNA analysis: not expressed as thalassemia in a Hb Tak/Hb E compound heterozygote. Hemoglobin 1998;22:45–52.

7 Charoenkwan P, Thanarattanakorn P, Chaovaluksakul S, Sittipreechacharn S, Saetung R, Sanguansermsri T: Hematological and molecular characterization of beta-thalassemia/Hb Tak compound heterozygote. Southeast Asian J Trop Med Public Health 2003;34:415–419.

8 Tanphaichitr VS, Viprakasit V, Veerakul G, Sanpakit K, Tientadakul P: Homozygous hemoglobin Tak causes symptomatic secondary polycytemia in a Thai boy. J Ped Hematol Oncol 2003;25:261–265.

9 Wood WG: Increased Hb F in adult life. Baillieres Clin Haematol 1993;6:177–213.

10 Fucharoen S, Pengjam Y, Surapot S, Fucharoen G, Sanchaisuriya K: Molecular characterization of $(\delta\beta)^0/\beta^0$-thalassemia and $(\delta\beta)^0$-thalassemia/Hb E in Thai patients. Eur J Haematol 2001;67:258–262.

11 Panyasai S, Fucharoen S, Surapot S, Fucharoen G, Sanchaisuriya K: Molecular basis and hematologic characterization of δβ-thalassemia and hereditary persistence of fetal hemoglobin in Thailand. Haematologica 2004;89:777–781.

12 Sanchaisuriya K, Chunpanich S, Fucharoen G, Fucharoen S: Multiplex allele specific PCR assay for differential diagnosis of Hb S, Hb D-Punjab and Hb Tak. Clin Chim Acta 2004;343:129–134.

13 Srivorakun H, Fucharoen G, Sae-ung N, Sanchaisuriya K, Ratanasiri T, Fucharoen S: Analysis of fetal blood using capillary electrophoresis system: a simple method for prenatal diagnosis of severe thalassemia diseases. Eur J Haematol 2009;83:57–65.

14 Boonsa S, Sanchaisuriya K, Fucharoen G, Wiangnon S, Jetsrisuparb A, Fucharoen S: The diverse molecular basis and hematologic features of Hb H and AEBart's diseases in northeast Thailand. Acta Haematol 2004;111:149–154.

15 Sae-ung N, Fucharoen G, Sanchaisuriya K, Fucharoen S: α^0-Thalassemia and related disorders in northeast Thailand: a molecular and hematological characterization. Acta Haematol 2007;117:78–82.

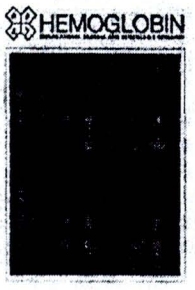
16 Nuntakarn L, Fucharoen S, Fucharoen G, Sanchaisuriya K, Jetsrisuparb A, Wiangnon S: Molecular, hematological and clinical aspects of thalassemia major and thalassemia intermedia associated with Hb E-β-thalassemia in northeast Thailand. Blood Cells Mol Dis 2009;42:32–35.

17 Yamsri Y, Sanchaisuriya K, Fucharoen G, Sae-ung N, Ratanasiri T, Fucharoen S: Prevention of severe thalassemia in northeast Thailand: 16 years experience at a single university center. Prenat Diagn 2010;30:540–546.

18 Teawtrakul N, Sirijirachai C, Chansung G, Fucharoen G: Compound heterozygous Hb Tak/Hb E causes secondary erythrocytosis in a Thai boy. Hemoglobin 2010;34:165–168.

19 Papassotiriou I, Traeger-Synodinos J, Marden MC, Kister J, Liapi D, Prome D, Stamoulakatou A, Wajcman H, Kanavakis E: The homozygous state for Hb Crete [β129 (H7) Ala→Pro] is associated with a complex phenotype including erythrocytosis and functional anemia. Blood Cells Mol Dis 2005;34:229–234.

Hemoglobin



Hemoglobin Phimai [beta72(E16)Ser->Thr]: a novel beta-globin structural variant found in association with Hb Constant Spring in pregnancy

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Keywords:	Hb Phimai, Hb Constant Spring, Hemoglobinopathies, Multiplex PCR

Hemoglobin Phimai [β72(E16)Ser→Thr]: a novel β-globin structural variant found in association with Hb Constant Spring in pregnancy

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ABSTRACT

A Thai pregnant woman with α- and β- hemoglobinopathies is described. Initial Hb analysis revealed an unknown variant with HPLC elution pattern similar to Hb Hope. Subsequent DNA-based diagnostics revealed that she was a carrier of Hb Constant Spring, and a novel β-globin chain variant (β codon 72 AGT→ACT or Ser→Thr) which we named Hb Phimai. Her hematological findings, and a simple DNA test for differential diagnosis of Hb Phimai and Hb Hope are presented.

Key words: Hb Phimai, Hb Hope, Hb Constant Spring, Hemoglobinopathies, Multiplex PCR

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