Expression of β^E- and γ-globin genes in infants heterozygous for hemoglobin E and double heterozygous for hemoglobin E and α-thalassemia

This study described the expression of β^E-globin in newborns heterozygous for HbE. Despite the lower level of HbE, the pattern of β^E-globin gene expression was similar to β^E-globin because the increase in HbE and HbA reached the peak level at the same time. A delayed decline of HbF was observed.

The prevalence of hemoglobin (Hb) E, the most common hemoglobinopathy in Southeast Asia, is 10-60% in Thailand.\(^1\) Substitution of guanine by adenine in codon 28 of the β-globin gene activates an adjacent cryptic splice site in codon 25 resulting in a mild β-thalassemia, although the existing correctly spliced mRNA can be translated to the full-length β^E-globin chains.\(^2\)

The slower switching process from HbF to HbA during the perinatal period of newborns heterozygous for β^E-thalassemia compared to normal controls has been observed.\(^3\) However, there is no report on the fetal-to-adult globin switch in HbE heterozygotes. To address this, hemoglobin type of cord blood from 256 newborns residing in the northeast of Thailand, where HbE is prevalent, was analyzed using an automated HPLC (VARIANT\textsuperscript{TM}, Bio-Rad, USA).\(^4\) Newborns who have HbE were followed every 2-3 months until one year of age. The study protocol was approved by the Institutional Review Boards of Mahidol University (Bangkok, Thailand), and the maternal consent was sought prior to taking cord blood and follow-up samples. All statistical analyses were carried out using the unpaired t-test.

Of the 256 cord blood samples, 153 (59.8%) had a normal Hb type, F+A, 6 had Hbs F+A+Bar’s (2.3%) and 97 samples (37.9%) were found to have an abnormal Hb type, F+A+Bart’s (2.3%) and 97 samples (37.9%) were found to have an abnormal Hb type, F+A+Bart’s (2.3%). Co-inheritance of various genotypes of α-thalassemia, including α^-thalassemia, α^-thalassemia and Hb Constant Spring (CS), accounting for 23%, was also detected among HbE heterozygotes using HPLC and a PCR-based technique.\(^5\) Due to family mobility and loss of contact, only 49 HbE heterozygous infants could be followed until one year old.

As shown in Figure 1, the pattern of β^-globin gene expression was similar to that of the normal β^-globin gene. At birth the level of HbE was low, ranging from 0.1-0.3%. The amount of HbE increased sharply at 2-6 months post-parturum and reached a peak level of 29.5±0.4%, at 6 months post-parturum.

In comparison to HbE heterozygotes, infants doubly heterozygous for HbE and α^-thalassemia had a significantly lower level of HbE throughout the neonatal period (p<0.01, Figure 1A). In contrast the levels of HbE at 10 months in infants doubly heterozygous for HbE and α^-thalassemia or HbCS were only slightly lower than those of HbE heterozygous infants. This is consistent with the data from adult double heterozygotes.\(^3\)

Electrostatic interactions are an important determinant of hemoglobin assembly. The reduction of HbE in newborns doubly heterozygous for α^-thalassemia is due to a reduced ability of positively charged β^-globin to assemble with α^-globin.\(^10\) This also explains why the highest level of HbA was at 10 months while HbE was at 6 months (Figure 1B). After 6 months, as HbF declines, there are more α^-globins to assemble with β^- and the former binds to α^-globin better, leading to a slightly increased HbA at 10 months.

The level of HbF in HbE heterozygous infants decreased sharply during the first four months of life (Figure 2). However, in comparison to normal infants, the levels of HbF were 3-4 times higher than those of normal infants of the same age indicating a delay in the decrease of HbF levels (p<0.01, Figure 2). At 3 months the amount of HbF in HbE heterozygote was significantly higher than that of normal infants, 34.5±10.4 vs 8.47±5.90%, and decreased to near normal levels at 12 months. This is consistent with findings observed in β^-thalassemia.\(^3\)

We suggest that the presence of β^-gene influences the delayed decrease and higher levels of HbF in newborns heterozygous for HbE. The α^-acting sequences in the individual globin gene and the LCR serve as templates for transcription factor binding. These proteins have different functions which include mediation of LCR and...
promoters interaction. Since βE-globin gene acts as a β+-thalassemia, the abnormal gene expression may delay the switch from γ- to β-globin and results in a higher production of HbF in newborns heterozygous for HbE.

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