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ORIGINAL ARTICLE

TOWARDS A PREVENTION PROGRAM FOR β-THALASSEMIA. THE MOLECULAR SPECTRUM IN EAST JAVA, INDONESIA

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□ *Defining the spectrum of specific thalassemia mutations is an important issue when planning prevention programs in large multi ethnic countries as is Indonesia. In a first attempt to define the prevalence of the common mutations in East Java we selected a cohort of 17 transfusion-dependent patients attending the Dr. Soetomo Hospital, Surabaya, Indonesia. After basic diagnostics we performed direct DNA sequencing for all β-globin genes. The results obtained on 34 independent chromosomes revealed the following prevalence rates: c.79 G>A p. Glu27Lys (Hb E) 47.0%; c.92+5G>C (IVS-I-5 G>C) 20.6%; c.109_110 delC p.Pro37Leu fs X7 [codon 35 (–C)] 17.6%; c.46del T p.Trp16Gly fsX4 [codon 15 (–T)] 5.9%; c.126_129delCTTT p. Phe42Leu fs X19 (codons 41/42) 2.9%; c.316-197 C>T [IVS-II-654 (C>T)] 2.9%; c* 112 A>G (PolyA) 2.9%. Our preliminary results show that the distribution of the prevalent mutations in our cohort is quite homogeneous but with different forms than previously reported. This indicates that more studies on a larger scale and in different geographical areas are needed to refine our provisional results and to characterize the molecular background of the disease in the whole country.*

Keywords Prevention, β-Thalassemia (β-thal), East Java, Indonesia

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INTRODUCTION

Since 7% of the world's population carries a hemoglobinopathy trait, these genetic disorders are the most common worldwide (1). Hemoglobinopathies are caused by mutations affecting the globin genes, causing a structural modification of the gene product [abnormal hemoglobins (Hbs)] or compromising the expression of the gene (thalassemias). Hemoglobinopathies are recessive disorders, therefore carriers are usually healthy or affected by a mild but chronic microcytic anemia. Moreover, carriers are protected against child mortality due to malaria, thus the prevalence of thalassemia traits in Indonesia is high and variable from area to area (3–8%) (2). Defining the spectrum of the specific thalassemia mutations is an important issue when planning prevention programs in large multi ethnic countries as in Indonesia where different mutations are expected to be prevalent in different areas or ethnic groups. Therefore, in a first attempt to define the prevalence of the common mutations in East Java we selected for molecular analysis a cohort of 17 transfusion-dependent patients attending the Dr. Soetomo Hospital, Surabaya, Indonesia.

MATERIALS AND METHODS

Cohorts and Basic Diagnostics

In total, we were able to collect blood samples from 60 individuals (patients and families) of whom 17 were transfusion-dependent patients from the Children Health Department at the Dr. Soetomo Hospital, Surabaya, Indonesia. The patients and their families all gave informed consent. All of them were diagnosed at the clinical and hematological level in Surabaya, in collaboration with the Medical Genetics Department, Wijaya Kusuma Surabaya University (UWKS), and in collaboration with the Hemoglobinopathy Diagnostic Laboratory of the Department of Human and Clinical Genetics at the Leiden University Medical Centre, Leiden, The Netherlands.

Patients and carriers were diagnosed by clinical examination and by measuring red cell indices on a semiautomatic counter (Micros 60; ABX Diagnostics, Montpellier, France), by excluding iron deficiency and by observing their erythroid cell morphology. The Hb fractions were separated and measured by high performance liquid chromatography (HPLC) (VARIANT II™; Bio-Rad Laboratories, Hercules, CA, USA) and on capillary electrophoresis (CE) (Capillarys®; Sebia, Lisses, France) as previously described (3).

Molecular Analyses

DNA was previously isolated using QIAamp DNA Kits following the instructions of the manufacturer (QIAGEN, Valencia, CA, USA). Molecular analysis was obtained in cooperation with the Hemoglobinopathy Diagnostic Laboratory at Leiden, The Netherlands.

Three segments of the β-globin gene that covered all mutations were amplified with the primers routinely used for β gene sequencing on an ABI PRISM™ 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using an ABI PRISM® Big Dye Terminator v2.0 Cycle Sequencing Kit (Applied Biosystems) as previously described (4).

RESULTS

Direct β gene sequencing of all 17 patients revealed the Hb E (c.79 G>A p. Glu27Lys)/β-thal genotype in 16 cases (94.1%). The frequency figures calculated on the 34 independent chromosomes revealed the following prevalence rates: Hb E 47.0%; c.92+5G>C (IVS-I-5 G>C) 20.6%; c.109_110 delC p.Pro37Leu fs X7 [codon 35 (–C)] 17.6%; c.46del T p.Trp16Gly fsX4 [codon 15 (–T)] 5.9%; c.126_129delCTTT p. Phe42Leu fs X19 (codons 41/42) 2.9%; c.316-197 C>T [IVS-II-654 (C>T)] 2.9%; c*112 A>G (PolyA) 2.9%. The data are summarized in Tables 1 and 2.

DISCUSSION

Surabaya is the capital city of East Java with a busy harbor favoring migrations and ethnic mixtures (Figure 1). Consanguinity is not a common issue in this area, therefore we are mainly concerned with the existing and changing patterns of β-thal mutations in Surabaya, Indonesia. The initial steps for any prevention program are the determination of the local spectrum of

TABLE 1 Summary of the β Genotypes Characterized in the 17 Cases Presented as Transfusion-Dependent Patients

Genotype	Samples ($n = 17$)	%
c.79 G>A p. Glu27Lys (Hb E) + c.92+5G>C [IVS-I-5 (G>C)]	7	41.2
c.79 G>A p. Glu27Lys (Hb E) + c.109_110 delC p.Pro37Leu fs X7 [codon 35(–C)]	6	35.3
c.79 G>A p. Glu27Lys (Hb E) + c.46del T p.Trp16Gly fsX4 [codon 15 (–T)]	2	11.8
c.79 G>A p. Glu27Lys (Hb E) + c.126_129delCTTTp.Phe/42Leu fs X19 (codons 41/42)	1	5.9
c.316-197 C>T [IVS-II-654 (C>T)] + c.*112 A>G (Poly A)	1	5.9

TABLE 2 Prevalence of the β Gene Defects in the 34 Independent Chromosomes of the Transfusion-Dependent Cases

Mutation	Number of Alleles	Percentage
c.79 G>A p. Glu27Lys (Hb E)	16	47.0
c.92+5G>C [IVS-I-5 (G>C)]	7	20.6
c.109_110 delC p.Pro37Leu fs X7 [codon 35 (–C)]	6	17.6
c.46del T p.Trp16Gly fsX4 [codon 15 (–T)]	2	5.9
c.126_129delCTTTp.Phe42Leu fsX19 (codons 41/42)	1	2.9
c.316-197 C>T [IVS-II-654 (C>T)]	1	2.9
c.*112 A>G (Poly A)	1	2.9

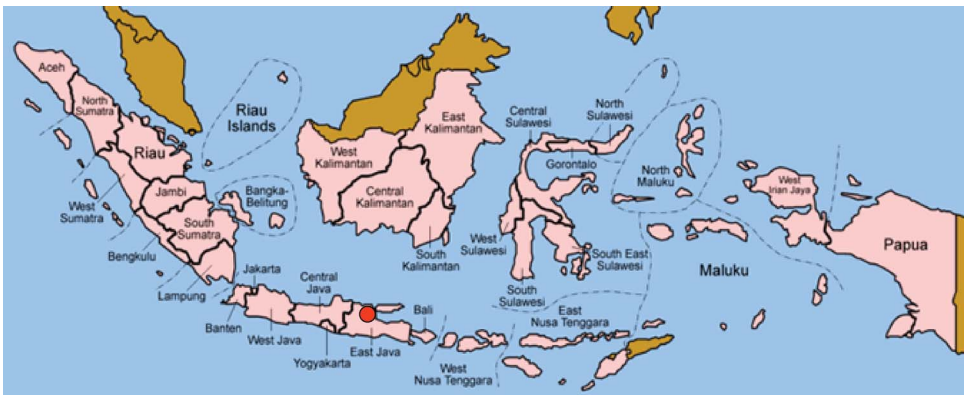

FIGURE 1 Map of the islands comprising Indonesia. The capital city, Surabaya, is indicated by a red spot in the middle of East Java.

β-thal mutations and the feasibility of prenatal diagnosis. Therefore, we have started our survey by analyzing the genotypes of local transfusion-dependent patients.

Our preliminary results show that 16 out of the 17 transfusion-dependent thalassemia patients in Surabaya, Indonesia were compound heterozygotes for Hb E/β-thal (94.1%). It should be noticed that transfusion dependency in Hb E/β-thal might become delayed when the β-thal defect is mild and when the patients present with a coexisting α-thal, a trait also common is Southeast Asia. In spite of the fact that the Hb E mutation is an abnormal Hb with a β^+-thal phenotype, expressed at about 20%, transfusion usually becomes necessary within the first decade of life because of progressing and life-threatening anemia.

The Hb E mutation has been strongly selected during evolution with peaks of high frequency in several parts of Southeast Asia, and in Malaysia in particular. A previous study by Lie-Injo *et al.* (4) on 36 Indonesian patients from West Java reported 13 patients who were carriers of the β^E allele (18%),

while the remaining β-thal mutations were distributed as follows: IVS-I-5 (G>C) 44.0%; IVS-II-654 (C>T) 9.7%; codons 41/42 and codon 35, both at 1.4%. Both cohorts investigated in these studies were small and possible ethnic differences and/or patient selection bias should be taken into consideration. However, the higher prevalence of the Hb E allele in the east of the country, possibly more related to Malaysia rather than West Java (48.5 compared to 18.0%) seems significant, as well as the distribution of the different types of β-thal mutations.

In conclusion, our data confirm that: a) the Hb E allele is predominant in East Java; b) different spectra of mutations can be expected from different areas and different ethnic groups in Indonesia, c) a common polymerase chain reaction (PCR) amplification with allele-specific oligonucleotide probes could be considered sufficient to define the molecular spectrum of the prevalent β-thal mutations in Indonesia as reported by Tan *et al.* (5). However, all samples in this study were characterized in The Netherlands where the methods of choice were sequencing, gap-PCR and multiplex ligation-dependent probe amplification (MLPA) (6). This was to address the huge heterogeneity of β-thal mutations, precluding the danger of being unable to characterize mutations, especially during prenatal diagnosis. In fact, the heterogeneity of mutations within geographical regions is becoming a worldwide problem due to the increasing incidence of population migration, even in traditionally endemic countries which originally may have had a limited number of specific local mutations.

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Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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