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Short Communication

DELETION OF TWENTY SEVEN NUCLEOTIDES WITHIN EXON 11 OF THE BAND 3 GENE IDENTIFIED IN OVALOCYTOSIS IN LOMBOK ISLAND, INDONESIA

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Summary This study reports the molecular characterization of ovalocytosis in Lombok Island, Indonesia. The analysis of genomic DNA by polymerase chain reaction shows that all 21 ovalocytotic individuals have two amplified products of different size from a region encompassing exon 11 of the band 3 gene. The sequence of the larger product matched perfectly with that of normal individuals. In the sequence of the smaller product, 27 nucleotides within exon 11 were deleted. The heterozygous presence of the deletion identified in other parts of Southeast Asia was confirmed in patients with ovalocytosis in an isolated island of eastern Indonesia.

Key Words ovalocytosis, erythrocyte, band 3, deletion

Southeast Asian ovalocytosis (SAO) is a hereditary form of elliptocytosis resulting in rigid, oval-shaped erythrocytes resistant to invasion by malarial parasites. The molecular basis for SAO was recently identified as a heterogeneous presence of an altered erythrocyte band 3 protein which lacked 9 amino acids (residues 400-408) at the boundary between the cytoplasmic and membrane domains (Jarolim *et al.*, 1991). The same mutation has been described in ovalocytotic individuals of Mauritius (Schofield *et al.*, 1992), Malaysia, the Philippines, and Papua New Guinea (Tanner *et al.*, 1991; Mohandas *et al.*, 1992). The same mutation was again identified in individuals with ovalocytosis from a western island in Indonesia (Takeshima *et al.*, 1994). However, no mutation study

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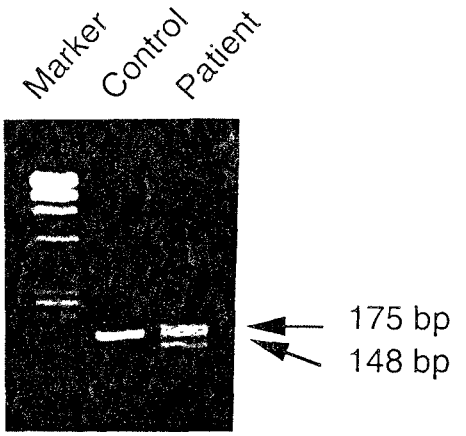

Fig. 1. Amplification of genomic DNA encompassing exon 11. The exon 11 encompassing region was amplified by using a set of primers (forward: 5′-GGGCCCAGATGACCCTCTGC-3′; reverse: 5′-GCCGAAGGTGATGGCGGGTG-3′) and separated by 3% agarose gel electrophoresis (Takeshima *et al.*, 1994). Only one band corresponding to 175 bp was visualized after amplification of the control DNA. The ovalocytosis DNA gave this same product as well as the smaller 148 bp product.

has ever been conducted among affected individuals from the eastern part of Indonesia. This paper describes the results of molecular analysis done on ovalocytotic individuals of a different Indonesian ethnic source. Twenty-one non-related individuals from Lombok Island in eastern Indonesia were diagnosed to have ovalocytosis based on the criteria used by Takeshima *et al.* (1994). DNA samples were extracted from blood cells of these subjects. Molecular analysis of the band 3 gene was the performed as described before (Takeshima *et al.*, 1994). A 175 bp long region spreading from nt. 1098 to nt. 1272 of band 3 protein cDNA (numbering was based on that used by Tanner *et al.* (1988)) was amplified (Jarolim *et al.*, 1991). Only one band corresponding to 175 bp in length was visualized on the agarose gel after ethidium bromide staining for the control DNA (Fig. 1). Meanwhile, 2 narrowly separated bands were obtained from the ovalocytosis DNA. One was slightly smaller than the single band derived from the control and the other comigrating with the control (Fig. 1). All 21 subjects revealed the same results.

The sequencing result disclosed that the smaller amplified product had a deletion of 27 nucleotides extending from nt. 1198 to 1224. There was no other nucleotide difference noted from the wild sequence (Fig. 2). The deleted 27 nucleotides corresponded to codons 400 to 408 of the band 3 protein. These findings confirmed that individuals with ovalocytosis from Lombok Island are also heterozygous for the abnormal band 3 protein.

It has been reported for both Papua New Guinea and Bangka Island in

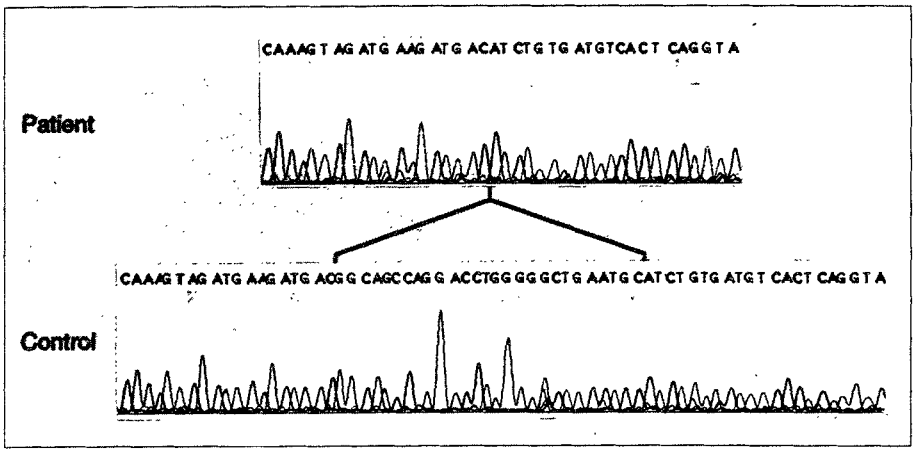

Fig. 2. Nucleotide sequence of part of the amplified product. After inserting the amplified product into pT7Blue(R)T-vector, the sequence of the subcloned DNA was determined using an atuomated DNA sequencer (model 373A; Applied Biosystems, Foster City, CA). Two types of DNA clone were obtained from PCR-amplified ovalocytosis samples. One of them corresponded to control band 3 (lower). The other type of clone which represents the abnormal ovalocytic band 3 differed from the normal because of the deletion of 27 nucleotides (upper).

western Indonesia that a deletion of 27 nucleotides in the band 3 gene is responsible for ovalocytosis. A sea of information can still be obtained for molecular genetic studies on ovalocytotic individuals from the several islands between this regions. Lombok Island, located in the eastern part of Indonesia, is still an endemic malarious region. It is isolated from the Island of Java. We focused our molecular analysis of ovalocytosis on this isolated island. True enough, we identified the same mutation in all 21 cases of ovalocytosis.

Hemoglobinopathies (including α- and β-thalassemias and sickle cell anemia), glucose 6 phosphate dehydrogenase deficiency, and ovalocytosis are very common genetic diseases in endemic malarious area. Because genetic mutations can provide resistance to malarial infection, malarial selection is suggested to determine the frequency of genetic disease in human population (Flint *et al.*, 1993). Our results confirmed that ovalocytosis in an isolated Indonesian island presents with the same amino acid deletion of the band 3 protein as reported in other countries (Tanner *et al.*, 1991; Mohandas *et al.*, 1992; Schofield *et al.*, 1992; Takeshima *et al.*, 1994). This is the second example of a genetic disease that is common in endemic malarious regions and is caused by only one kind of mutation. If it is true that only one mutation is common in areas from Melanesia to Mauritius, what factor may provide such phenomenon? It is conceivable that SAO has a single origin and distribution of SAO goes alongside with the

migration of people (Schofield *et al.*, 1992; Liu *et al.*, 1994).

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