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TWENTY SEVEN NUCLEOTIDE DELETION WITHIN EXON 11 OF THE ERYTHROCYTE BAND 3 GENE IN INDONESIAN OVALOCYTOSIS

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Summary We here report the molecular characterization of an Indonesian ovalocytosis. The analysis of genomic gene by polymerase chain reaction shows that the individual has two amplified products from a region encompassing exon 11 of the erythrocyte band 3 gene. The sequence of the larger product matched completely with that of normal individuals. In the sequence of the smaller product, 27 nucleotides within exon 11 disappeared. The deletion removes a total of nine amino acids in the boundary of cytoplasmic and membrane domains of band 3 protein, a membrane anion transporter protein. This is the first report to confirm the heterogeneous presence of an altered membrane band 3 protein in Indonesian ovalocytosis.

Key Words ovalocytosis, erythrocyte, band 3, Indonesia

Southeast Asian ovalocytosis (SAO) is a hereditary form of elliptocytosis resulting in rigid, oval-shaped erythrocytes resistant to invasion by malaria parasites. The molecular basis for SAO was recently identified as a heterogeneous presence of an altered erythrocyte band 3 protein, which lacked nine amino acids (residues 400–408) at the boundary between cytoplasmic and membrane domains (Jarolim *et al.*, 1991). As far as we know, the same mutation has been described in ovalocytosis not only from Malaysia, the Philippines, and Papua New Guinea (Tanner *et al.*, 1991; Mohandas *et al.*, 1992) but also from Mauritius (Schofield *et al.*, 1992b). However, no report describes results of molecular analysis on the band 3 gene in Indonesia, where ovalocytosis has been reported to be very common (Sofro, 1986). In order to clarify the mutation responsible for Indonesian ovalocytosis, we analyzed the erythrocyte band 3 gene. We describe here the result of

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molecular analysis of hereditary ovalocytosis from a different ethnic source, an Indonesian.

The Indonesian case was diagnosed to be ovalocytosis, because examination of his peripheral blood smear disclosed 100% of red blood cells were ovalocytic. Blood obtained from the case was spotted on a filter paper and the sample was mailed to Japan. DNA sample was extracted from dried blood cells by standard phenol/chloroform extraction methods and used as a template for polymerase chain reaction (PCR) amplification. To determine a mutation in the band 3 gene, we first tried to screen the same deletion mutation as reported before (Jarolim *et al.*, 1991). For this, 175 bp long region spreading from nt. 1098 to 1272 of band 3 protein cDNA (numbering as by Tanner *et al.*, 1988) was amplified (Jarolim *et al.*, 1991). From normal DNA only one band corresponding to 175 bp length was visualized on the agarose gel after ethidium bromide staining (Fig. 1). In contrast, two narrowly separated bands were obtained from Indonesian ovalocytosis case, *i.e.*; the one slightly smaller than the single band derived from a control, the other comigrating with the control (Fig. 1). These results showed that the index case is heterogeneous for the band 3 gene and strongly suggested that one gene contains a deletion in the amplified region.

As the size difference of the amplified products suggests a presence of a deletion in the gene, the amplified products were sequenced. The amplified DNA was directly subcloned into pT7Blue T-vector (Novagen, Madison, WI). The sequences of inserted DNA from nine or ten clones were determined by an automatic DNA sequencer (model 373A: Applied Biosystems Inc., Foster City, CA) using the Taq dyeprimer cycle sequencing kit (Applied Biosystems Inc.). One of them, corresponding to normal size product, disclosed the sequence completely matched with that of wild type (Fig. 2). The other type of clone, which represent the smaller amplified product, had a 27 nucleotides deletion extending from nt. 1198 to 1224

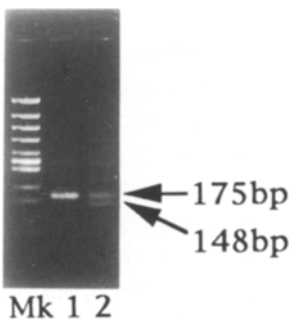

Fig. 1. Amplification of genomic DNA encompassing exon 11. Only one band corresponding to 175 bp was visualized after amplification of normal DNA (lane 1) while the Indonesian ovalocytosis DNA gave this product as well as the smaller 148 bp product. The PCR was carried out as described before (Matsuo *et al.*, 1991). The amplified DNA fragment was separated by 3% agarose gel electrophoresis and photographed after ethidium bromide staining.

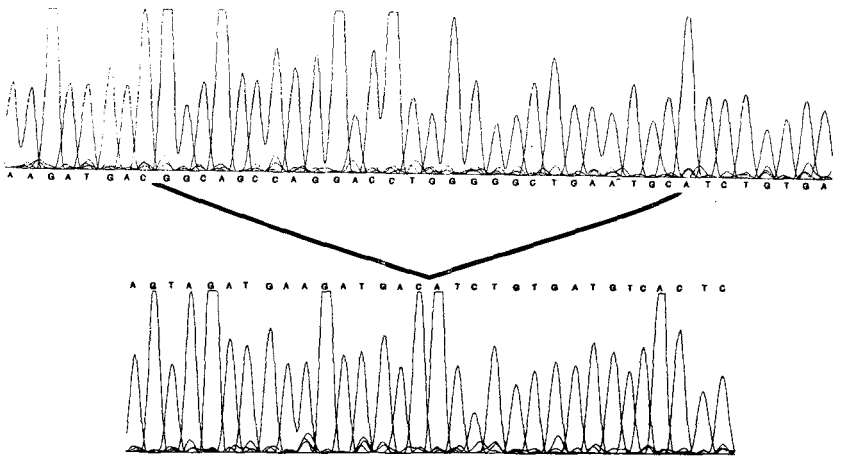

Fig. 2. Nucleotide sequence of part of the amplified product. Two types of DNA clone were obtained from PCR-amplified ovalocytosis sample. One of them corresponded to normal band 3 (upper). The other type of clone, which represents the abnormal ovalocytic band 3, differed from normal and 27 nucleotides of GCA TTC AGC CCC CAG GTC CTG GCT GCC disappeared (lower).

and no other nucleotide difference from the wild sequence (Fig. 2). The deleted 27 nucleotides of GCA TTC AGC CCC CAG GTC CTG GCT GCC corresponded to codons 400 to 408 of band 3 protein. These findings confirmed that the Indonesian ovalocyte is also heterozygous for the abnormal band 3 protein that has nine amino acids deletion.

Band 3 protein, an anion-exchange protein, is composed of two domains: an N-terminal cytoplasmic domain and a membrane domain. It is the membrane domain spanning the bilayer 14 times that is responsible for anion-exchange activity. The deleted segment found in Indonesian ovalocytosis represents the interface between the N-terminal cytoplasmic part of the protein and the first transmembrane segment. And this region comprises a highly conserved sequence element and corresponds to the hinge which adapt to the displacement of the ankylin (Schofield *et al.*, 1992b). It is experimentally clarified that the biophysical consequences of this mutation are a marked decrease in lateral mobility of band 3 in the membrane, and an increase in membrane extensional rigidity (Mohandas *et al.*, 1992). And it is also shown that the protein having nine amino acids deletion is defective in anion transport activity (Schofield *et al.*, 1992a).

One nucleotide change from A to G at nt. 166 of band 3 cDNA, which is found in a asymptomatic band 3 variant known as the Memphis variant (Jarolim *et al.*, 1992), has been reported to link with a deletion of codons 400–408 of band 3 protein (Jarolim *et al.*, 1991; Schofield *et al.*, 1992b; Mohandas *et al.*, 1992). In the index case, the region around nt. 166 was amplified using a set of primers (5′-GAGGCAACAGCCACAGACTA-3′ and 5′-CTCCTGCAGCTCCACATAGA-3′) and was

sequenced. The case was found to be heterogeneous for A and G nucleotides at nt. 166 (data not shown). This suggested that the deletion mutation is also linked to the Memphis variant.

In malaria endemic areas, three hemoglobinopathies including α- and β-thalassemias and sickle cell anemia, glucose 6 phosphate dehydrogenase (G6PD) deficiency, and ovalocytosis, are very common genetic diseases. Because these genetic mutations provide resistance to malaria infection for individuals with the mutation. And malarial selection is suggested to determine the frequency of genetic disease in human population (Flint *et al.*, 1993).

Mutations defined in three genetic diseases, *i.e.* α- and β-thalassemias and G6PD deficiency, respectively, are quite heterogeneous and differ from region to region where the case is found. In contrast to these, the genetic basis for sickle cell anemia is a same single nucleotide mutation of the β-globin gene, although it is distributed in Africa, Middle East, and India. Our result confirmed that Indonesian ovalocytosis is resulted from the same amino acid deletion of band 3 protein as reported in other countries. And this is the second example of genetic disease giving resistance to malaria infection that is caused by only one kind of mutation. If it is true that only one mutation is spread from Melanesia to Mauritius, what factor may provide such phenomenon? It is conceivable that there has been a single origin of SAO and SAO is distributed along with migration of people. Considering that inheritance of nine amino acids deletion of band 3 protein is linked with that of Memphis variant which is one of polymorphisms located at 5′ end of the band 3 gene, the single origin theory is highly plausible. Other possibility is that unknown factor predisposing the same deletion may be present around the deletion site of the band 3 gene (Sommer and Ketterling, 1993). But it needs further study to be qualified.

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