The Homozygous State for the Band 3 Protein Mutation in Southeast Asian Ovalocytosis May Be Lethal

To the Editor:

Southeast Asian Ovalocytosis (SAO) is an asymptomatic trait characterized by oval red blood cells (RBCs) that are rigid and resist invasion by several malaria parasite strains. Our laboratory and others have reported that the underlying molecular defect involves a deletion of nine codons (codons 400-408) in the erythroid band 3 gene. Band 3 protein, the major integral protein of the RBC membrane, consists of two domains with distinct structure and function. The N-terminal cytoplasmic domain provides a binding site for ankyrin that anchors the spectrin-based membrane skeleton to the membrane. The transmembrane domain, composed of multiple membrane spanning helices, is the site of transport of anions across the RBC membrane. The deletion of nine amino acids in SAO band 3 resides at the boundary of the cytoplasmic domain and the first transmembrane segment of band 3 protein that is tightly linked with the band 3-Memphis, a common band 3 polymorphism with abnormal electrophoretic mobility of band 3 caused by a substitution of lysine 56 by glutamic acid. At the functional level, the SAO band 3 mutation is characterized by a decreased transport of anions, a propensity to form linear aggregates in the membrane, an increased oligomerization of band 3, and an increased retention of SAO band 3 by the membrane skeleton. The latter two abnormalities are likely to account for a marked decrease in both lateral and rotational mobilities of the band 3 protein in the membrane, as well as the increased rigidity of the SAO red cell membrane.

We studied 105 SAO specimens either by tryptic digestion of band 3 (which detects the linked band 3-Memphis polymorphism) or by polymerase chain reaction amplification of the region of cDNA or genomic DNA that contains the site of the deletion. The blood samples originated from Papua New Guinea (18 samples), Malaysia (84 samples), and the Philippines (3 samples). All SAO samples tested in our laboratory were found to be heterozygous for the SAO band 3 deletion, which in all cases was linked with the band 3-Memphis polymorphism. Remarkably, the tested subjects was heterozygous for the SAO band 3 mutation. In some populations of Papua New Guinea and Malaysia, the frequency of SAO is as high as 40%. Therefore, the absence of homozygosity for the SAO band 3 mutation in the subjects under this study raises a possibility that the homozygous state is incompatible with life.

To examine this possibility, we studied 47 families from Malaysia that were divided into three groups (Table 1). In the first group of 18 families of the same ethnic background, both parents were negative for the SAO trait. The second group included 21 families, in which one parent had the SAO trait and was heterozygous for the SAO and band 3-Memphis mutations, whereas the other parent was normal. The third group contained 6 families in which both parents were heterozygous for the SAO and band 3-Memphis mutations. In the latter group, there were 35 offspring. Twelve of these offspring were available for testing. We found that 10 of them were heterozygous for the SAO band 3 mutation that was linked with the band 3-Memphis polymorphism, whereas 2 subjects did not carry either the SAO or the band 3-Memphis mutation. Specifically, none of these subjects was homozygous for the SAO band 3 mutation. In addition, an interview of mothers from the 6 families with both parents heterozygous for SAO mutation showed a higher frequency of miscarriages in this group than in the other two groups (Table 1). This finding further supports the conclusion that homozygosity for the SAO band 3 mutation may be incompatible with life.

The mechanism whereby the homozygous state for the SAO mutation is incompatible with life is not clear. Recent observations of about 50% decrease in anion transport of SAO RBCs suggest that the mutant allele is not functional in terms of anion transport. Thus, RBCs of homozygotes for the SAO mutation, or other cells expressing erythroid band 3 during embryonal or fetal life, may not be capable of chloride-bicarbonate exchange. Another possibility is that the homozygosity for the SAO mutations leads to an extensive agglutination of SAO band 3 in developing erythroblasts. The ensuing increase in membrane rigidity may preclude release of such RBCs from the bone marrow into the peripheral circulation.

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<th>Table 1. Miscarriages in SAO Families</th>
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<tr>
<td>No. of Families</td>
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<td>Two Parents Normal</td>
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<td>One Parent SAO Carrier</td>
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<tr>
<td>Both Parents SAO Carriers</td>
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<td>Miscarriages/total pregnancies</td>
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<td>Miscarriages (%)</td>
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REFERENCES


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SC Liu, P Jarolim, HL Rubin, J Palek, D Amato, K Hassan, M Zaik and P Sapak