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Natural history of Southeast Asian Ovalocytosis during the first 3 years of life

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ABSTRACT

Southeast Asian Ovalocytosis (SAO), the most common red cell membrane disorder found in the Far-East and Pacific rim, appears to be innocuous in man since it has been identified mostly in non-anemic healthy individuals. To further substantiate our previous observation that this condition might be symptomatic particularly in the neonatal period, we studied 1567 newborns from Southern Thailand where SAO is prevalent. Thirty-one babies (1: 50 with allele frequency of 0.01) have been identified with SAO and confirmed molecularly to carry a single defective *AE-1* (band 3) allele. These babies had significant anemia at birth due to hemolysis with 51.6% of them developing neonatal hyperbilirubinemia. Co-inheritance of common *UGT1A1* variants in such cases was not associated with their degree of jaundice. Interestingly, hematology data of these SAO babies became "normalized" in the first 3 years of life without further evidence of on-going and/or even "compensated" hemolysis.

Introduction

Southeast Asian Ovalocytosis (SAO) is an autosomal form of hereditary elliptocytosis which is widespread in Southeast Asia [1] and the South Pacific [2]. This results from the nine-amino-acid deletion of residues 400-408 due to 27 bp deletion of the band 3 (*AE1* or *SLC4A1*, *Anion-Exchanger 1 gene*) [3]. AE1 is the major transmembrane protein of the red blood cell and is important in maintaining both its internal milieu by passive anion transport, and its shape through attachment via ankyrin to spectrin in the cycloskeleton [4,5]. The majority of individuals with heterozygosity for SAO are asymptomatic with no clinically detectable hemolysis [6] with few exceptions [7]. The homozygous SAO deletion is thought to be lethal *in utero* in the embryonic state [1]. Previously, we proposed that SAO might play a role in anemia and hyperbilirubinemia in neonates; however, other possible causes of anemia could not be completely excluded due to the retrospective nature of the studies [8,9]. Therefore, we prospectively analyzed and followed 31 Thai SAO infants from birth to 3 years of age to demonstrate the natural history of this common condition.

Patients and methods

We conducted a prospective study between 1 February 2006 and 31 January 2007. This study was approved by the Institute Ethics Committee. Five milliliters of cord blood of the newborns whose parents or guardians provided informed consent were analyzed for CBC, reticulocyte number, blood group and G6PD screening test using standard techniques [10]. The diagnosis of SAO in the newborns was made based on criteria previously reported [8]. We collected their clinical data including gestational age, birth weight, placental weight, age at detection of pallor, age at detection of jaundice, the highest microbilirubin level and the duration of treatment by phototherapy. After birth, other causes of neonatal hemolysis in SAO neonates including ABO incompatibility, thalassemia and blood loss from feto-maternal hemorrhage (FMH) were excluded [11,12]. Each SAO baby underwent a full physical examination at the ages of 1, 2, 4, 6, 10, 12, 18, 24, 30 and 36 months to measure weight, height, the size of the liver and spleen together with CBC and reticulocyte count. Five known variants of the *AE1* gene: Codon 400–408 deletion (SAO 27 bp-deletion), Glycine 701 to Aspartic acid mutation (G701D), Alanine 858 to Aspartic acid mutation (A858D), Serine 773 to Proline mutation (S773P) and Glutamine 759 to Histidine mutation (Q759H), were determined as described [9,13]. In addition, three common uridine diphosphate glucuronosyltransferase 1A1 (*UGT1A1*) polymorphisms; TATA box (A(TA)7TAA) mutation (TA)7 at the promoter [14], Glycine

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71 to Arginine mutation (G71R) [15] and Phenylalanine 83 to Leucine mutation (F83L) [16] were molecularly identified.

Data analysis

Statistical analyses were conducted with STATA 7 software. Student's *t*-test was used to compare continuous data and chi squared test and Fisher exact test, where appropriate, to compare categorical data.

Results

From 1567 specimens of cord blood studied, we detected typical SAO morphology in 31 neonates indicating a prevalence of SAO in our population of 1:50 (allele frequency of 0.01). Four of them were associated with other conditions: two with Hb E trait, one with premature baby and one with FMH. Demographic data of 1,536 non-SAO newborns compared with SAO neonates are shown in Table 1. The SAO group had a significantly higher percentage of pallor at birth; 74.2% compared to 7.6% in the non-SAO group, and jaundice 67.7% compared to 12% in the non-SAO group (Table 1). SAO was not detected in neonates of Chinese descent. SAO neonates had a higher percentage of blood group A than that those found in non-SAO neonates. Hematology data after the first 24 h were compared between SAO and 802 non-SAO babies whose data were available and revealed Hb, Hct and RBC values to be significantly lower in neonates with SAO than in controls, in contrast to RDW and reticulocyte values, that were significantly higher. These findings together with greater jaundice suggest that significant hemolysis occurred in SAO neonates resulting in neonatal anemia. Our molecular analysis showed that all SAO cases carried only causative *AE-1* mutation. Co-inheritance of common *UGT1A1* variants in these SAO babies did not seem to aggravate the degree of jaundice; the highest bilirubin in individuals with wild type ($n=10$) and *UGT1A1* heterozygotes (14 of TA6/TA7, 2 of G71R and 1 of F83L) were 14.68 ± 3.17 and 14.61 ± 2.88 mg%, respectively. Only one case with homozygous for G71R variants had the highest bilirubin level (22.93 mg%) among the SAO babies. During three years follow-up, these SAO babies were unremarkable with normal growth and development. None has been found to have hepatosplenomegaly, hemolytic or anemic crisis during the first 3 years of life. Their Hb Hct and RBC values appeared to decline to the nadir at 2 months of age, compatible with normal physiological anemia of childhood (Fig. 1). Interestingly, these anemia parameters gradually increased afterwards to the normal range. However, this normalization did not seem to be a result of compensated hemolysis since the reticulocyte values remained constant throughout (Fig. 1).

Table 1
Clinical features of the newborn SAO and non-SAO babies.[a]

Characteristics	NB-SAO[b] ($n=31$)	NB-non-SAO[c] ($n=1536$)	*P*-value
Sex, M:F	16:15	785:751	0.955
Gestation age (weeks)	38.4 ± 1.5	38.7 ± 2.1	0.343
Birth weight (g)	$2{,}968\pm460$	$3{,}098\pm481$	0.136
Placenta weight (g)	613 ± 158	627 ± 113	0.545
Pallor	23 (74.2%)	116 (7.6%)	<0.001
Jaundice	21 (67.7%)	185 (12.0%)	<0.001
Highest MB[d] (mg%)	15.2 ± 3.4	12.3 ± 3.1	<0.001
MB > 15 mg%	14 (45.2%)	127 (17.2%)	<0.001
MB > 20 mg%	4 (12.9%)	8 (0.5%)	<0.001
Phototherapy	21 (67.7%)	188 (12.2%)	<0.001
Ethnicity			
Thai	26 (83.9%)	1377 (89.6%)	0.298
Muslim	5 (16.1%)	128 (8.3%)	0.123
Chinese	0	15 (1.0%)	0.580
Blood group:			
O	9 (29.0%)	578 (37.6%)	0.322
A	13 (41.9%)	364 (23.7%)	0.019
B	8 (25.8%)	474 (30.9%)	0.540
AB	1 (3.2%)	116 (7.6%)	0.363
Hematology after birth	NB-SAO[b] ($n=31$)	NB-non-SAO[e] ($n=802$)	
Age[f] (days)	1.97 ± 0.98	2.6 ± 1.0	
Hb (g/L)	141.3 ± 15.3	164.0 ± 23.5	<0.001
Hematocrit (%)	41.1 ± 4.49	49.4 ± 6.33	<0.001
RBC (x 10^{12}/L)	4.03 ± 0.53	4.78 ± 0.75	<0.001
MCV (fL)	102.7 ± 5.17	100.9 ± 6.75	0.14
MCH (pg)	35.4 ± 2.99	34.3 ± 2.80	0.029
MCHC (g/dL)	34.4 ± 2.06	33.9 ± 1.56	0.130
RDW (%)	17.5 ± 1.67	15.1 ± 1.94	<0.001
Reticulocyte (%)	12.22 ± 3.72	8.49 ± 4.31	<0.001
Reticulocyte (x 10^{10}/L)	48.75 ± 14.32	39.48 ± 19.00	0.010

[a] The data are presented as mean ± SD or (percent).
[b] Newborn with SAO.
[c] Newborn with non-SAO.
[d] MB = microbilirubin.
[e] Those with available hematology data.
[f] Age = age at determination (days), $P<0.05$ is considered as a statistically significant difference.

Discussion

In Southeast Asian countries, allele frequency of SAO among populations in Malaysia, Indonesia and Papua New Guinea were 0.01 [17], 0.01–0.09[18,19], and 0.01–0.15[19], respectively. Our result of 0.01 in Southern Thailand was similar to that of Malaysia which is our neighboring country on the mainland. It is widely thought that SAO is an asymptomatic trait without significant hemolysis. A large cohort in Papua New Guinean children has shown that children with SAO ($n=16$) had a hematological profile similar to that of their community controls ($n=225$) [20]. Infection with malaria appeared to have a greater effect in children with SAO as their Hb and RBC were lower than those of controls. However, there was no direct evidence of apparent hemolysis in such cases and this higher degree of malaria anemia might result from a selective loss of infested ovalocytes in SAO subjects. Only a few symptomatic cases with SAO have been reported with chronic hemolysis with increased reticulocytes; however it remains unclear whether the band 3 deletion was the only causative mutation in such cases [7]. Recently, several studies mainly derived from populations in our region have shown that compound heterozygous of SAO with other *AE-1* mutations; G701D, ΔV850, A858D, S773P and Q759H could result in hemolytic anemia even though all reported cases also developed autosomal recessive distal renal tubular acidosis (dRTA) and some might have had different red cell morphology [21]. However, stomatocytic ovalocytes remained present in some interactions (SAO 27 bp-deletion/A858D and SAO 27 bp-deletion/ΔV850). Therefore, it is important to determine whether neonatal anemia found in this study is not caused by interaction of SAO with other *AE-1* mutants, one of which (G701D) has previously been identified in our population [13]. Indeed, all SAO babies carried only the SAO 27 bp-deletion and our 3 yr-follow-up demonstrated that they all had normal growth with normal acid-base balance (data not shown) making the possibility of dRTA, and hence co-inheritance of another *AE-1* mutant(s), unlikely.

By excluding other possible cause of anemia at birth, we further confirmed that SAO might be "symptomatic" by causing "hemolysis" at birth. Why prenatal and neonatal conditions have set this "rigid" red cell to be on the verge of overt hemolysis remains to be elucidated, but it is likely that the pathophysiology might involve inelasticity by conformational or deformational change of band 3 and an inability of the deleted band 3 to transport anions [22]. Interestingly, an adaptive process in SAO babies has developed during their first 3 years of life since there was no further appearance of hemolysis. Their hematology showed normal pattern of "physiologic anemia of childhood" and complete return to the baseline as in controls at the same period. This warrants further study to explore what physical and biochemical properties of these ovalocytes might be additionally deranged at birth