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Letter to the Editor

Co-inheritance of Southeast Asian Ovalocytosis (SAO) and G6PD deficiency associated with acute hemolysis in a Thai patient

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To the Editor's

Southeast Asian Ovalocytosis (SAO) due to *SCL4A1* gene mutation is the most common inherited red blood cell (RBC) membrane disorders in man. The prevalence of SAO carrier is high in subtropical countries; 3% in the Southern part of Thailand [1] and greater in Malaysia, Indonesia and highest in Papua New Guinea (up to 35%) due to malarial pressure [2]. Previously, we have reported a series of neonatal hyberbilirubinemia associated with this genetic defect and their follow up during the first three years of life [3]. However, its role on susceptibility to acute hemolysis and clinical significance in adult life remains rarely documented.

A twenty-six-year-old Thai male from Central Thailand presented with a high-grade fever with jaundice for one day. He had no organ-specific symptoms, and his urine was darkening without dysuria. Three years earlier, he had a history of pyrexia with a sore throat and was identified with mild jaundice and transaminitis. His provisional diagnosis at that time was an acute viral infection with hemolysis secondary to glucose-6-phosphate dehydrogenase (G6PD) deficiency. However, the patient lost to follow up, and his definitive diagnosis of G6PD deficiency was not confirmed. His maternal grandfather had a similar clinical history of acute pyrexia and jaundice several times in the last five decades; however his definitive cause of recurrent hemolysis was not performed. On the physical examination, this patient was found with mild anemia with the icteric sclera, and others were unremarkable, including no hepatosplenomegaly. The laboratory evaluations from this patient and his mother (51 years old) and his grandfather (75 years old) are shown in Table 1. The presence of indirect hyperbilirubinemia with reticulocytosis (~7%) without severe anemia suggested a compensated hemolysis. Since the haptoglobin was not decreased, this is plausible with the mechanism of hemolysis due to red cell membranopathy occurring mainly at extramedullary sites, i.e. spleen and liver. This type of red cell destruction does not release free hemoglobin into the peripheral blood, so the haptoglobin cannot bind to and reduce its levels [4]. His peripheral blood smear revealed anisocytosis, poikilocytosis with numerous ovalocyte and stomatocytes with specific features of a longitudinal central slit or transverse ridge characteristic of SAO (Fig. 1). Similar red blood cell morphology was also observed in his mother and grandfather, and the patient's RBC showed decreased osmotic fragility suggesting increased rigidity (data not shown). However, we could not identify the presence of the 'bite cells' or red blood cell with hemoglobin leak or the presence of a Heinz body usually found in acute hemolytic crisis due to G6PD deficiency. The diagnosis of SAO was further confirmed as all three affected members having decreased mean fluorescence intensity (MFI; < 100%) using EMA binding assay and DNA diagnosis showed a 27 base pair in-frame deletion resulting in 9 amino acids deletion involving Ala400-Ala408 of the exon 11 of *SCL4A1* as described [3]. Further investigations including genetic analysis of candidate genes (as shown in Table 1) revealed that the patient and his grandfather also carried G6PD deficiency and his mother was a carrier and excluded other causes of hemolytic anemia such as α-and β-thalassemia and autoimmune hemolytic anemia (AIHA). Therefore, the final diagnosis in all three affected individuals was SAO (SLC4A1: c.1199_1225del(p.Ala400_Ala408del) and G-6-PD Mahidol: c.577G > A (p.Gly193Ser)).

The human anion exchanger 1 (AE1) or erythrocyte band 3 (EPB3) or CD233 or solute carrier family 4 (SLC4A1) gene is located on the long arm of chromosome 17(17q21-q22) encompassing approximately 20 kb and consisting 20 exons [2]. In erythrocytes, *SLC4A1* expresses an integral membrane protein with cytoplasmic N-terminal 40 kDa domain as binding sites for ankyrin and protein 4.2 maintaining the RBC's mechanical properties and integrity. The 55 kDa glycosylated C-terminal membrane-associated domain contains 12–14 membrane-spanning segments which function as a chloride/bicarbonate anion exchanger involved in CO_2 transport in the α-intercalated distal tubular cells and contributes to the urine acidification. Mutations of *SCL4A1* can lead to destabilizing RBC membrane causing hereditary spherocytosis (HS), ovalocytosis (HO), elliptocytosis (HE) and defective kidney acid transport; distal renal tubular acidosis (dRTA) [1].

The SAO inherits as autosomal dominant (AD) and results in tighter binding of band 3 to ankyrin, which increases the red cell membrane rigidity.[1] The rigid red cells loss deformability property and trapped in the splenic red pulp and leads to extravascular destruction. Although, homozygous SAO was thought to be lethal, [5] until recently it was confirmed as a hydrops was rescued by intrauterine transfusion [6]. This patient exhibited severe anemia from both hemolysis and dyserythropoiesis [6]. The SAO band 3 was detectable on peripheral RBC with increased permeability and fragility. However, heterozygous SAO individuals are generally asymptomatic without significant anemia suggesting a well-compensation (compensated hemolysis) [3]. It is possible that this physiological balance in SAO can be tipped off by other hemolytic factors added. A recent report in two unrelated probands by Chen et al. suggested that co-inheritance of SAO and β-thalassemia trait could result in significant hemolysis associated with gallstone formation and subsequent overt ineffective erythropoiesis leading to hemosiderosis and iron overload [7]. Our three individuals from the same

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Table 1
Summary of baseline hematology and laboratory evaluation in a family with Southeast Asian Ovalocytosis (SAO) with G6PD Mahidol (G6PD p.Gly193Ser).

Laboratory parameters (reference range/units)	Patient		Mother	Grandfather
	3 yrs. earlier	Present		
Hemoglobin (g/dL)	14.1	14.7	12.0	10.5
Hematocrit (%)	43	39.1	33.4	30.6
MCV (flL)	88	86.5	71.1	57.5
MCHC (g/dL)	38.0	37.6	35.9	34.3
RDW (%)	14.5	14.2	18.0	23.6
WBC (×1,000/μL)	72	18	7.1	17.1
Plt (×1,000/μL)	103	167	250	520
Total bilirubin (0–1 mg/dL)	2.56	3.19	1.72	1.93
Direct bilirubin (0–0.3 mg/dL)	0.67	0.91	0.32	0.41
Indirect bilirubin (0–1 mg/dL)	1.89	2.28	1.40	1.52
AST (15–37u/L)	138	135	36	13
ALT (30–65u/L)	96	49	58	23
Alkaline phosphatase (50–136 u/L)	41	72	75	61
Lactate dehydrogenase (100–190 u/L)	NA	184	189	237
Haptoglobin (36–195 mg/dL)	NA	39	40	68
Osmotic fragility (OF) (%hemolysis)	Decreased		ND	Decreased
Direct antiglobulin test	Negative		ND	ND
G-6-PD assay (10.7–14 IU/g Hb)	0.05		ND	ND
Hemoglobin typing (HPLC)	Hb A, A2 (3.2%)		Hb A, A2 (2.8%)	Hb A, A2 (3.0%)
EMA binding assay (MFI > 100%)	64.5		68.5	67.4
α- and β-globin genotype	αα/αα, β/β		αα/αα, β/β	αα/αα, β/β
SLC4A1 (AE-1) genotype	Heterozygous for c.1199_1225del			
G-6-PD genotype	Hemizygous for c.577G > A		Heterozygous for c.577G > A	Hemizygous for c.577G > A

Abbreviations: MCV; mean corpuscular volume, mean cell hemoglobin concentration; MCHC, red cell distribution width; RDW, WBC; white blood count, Plt; platelet count, AST; aspartate aminotransferase, ALT; alanine aminotransferase, HPLC; high pressure liquid chromatography, EMA; Eosin-5-maleimide, MFI; Mean fluorescence intensity. ND = not determined.

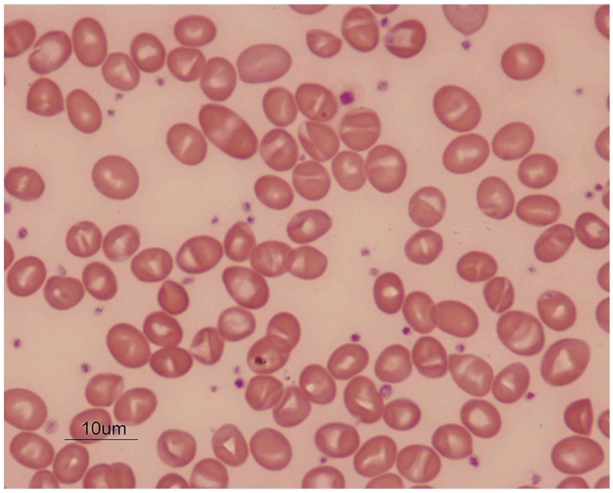

Fig. 1. Peripheral blood smear of a Thai patient with SAO and G6PD-Mahidol.

family further exhibit a significant hemolysis due to a confounding RBC enzymopathy. The G6PD-Mahidol variant (487 G > A) has recently been shown to be the most common G6PD variant found at the Northwestern Thailand-Myanmar border [8]. Interestingly, the authors found the enzymatic activity in a hemizygous male of G6PD-Mahidol to be < 10% changing this variant from the previous WHO Class III (moderate deficiency with 10–60% G6PD activity and hemolysis with stressors only) into Class II (severe deficiency with intermittent hemolysis). It has been suggested that the original classification of G6PD-Mahidol might be mistaken due to a lack of DNA confirmation in the past [8]. This result further supports that G6PD-Mahidol could significantly enhance the hemolytic risk of SAO heterozygous as described in our index family. In the endemic malarial region with a high frequency of RBC variants such as SAO, G6PD, thalassemia and other hemoglobinopathies such as Thailand, an interaction of these polymorphisms could result in a complex genotype-phenotype interaction leading to unusual clinical presentation and long-term complications that require careful medical evaluation and management.

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