Original Article
Hereditary spherocytosis combined with glucose-6-phosphate dehydrogenase deficiency in a Chinese family

Shiyue Ma[1*], Xuelian Deng[1*], Lin Liao[1], Zengfu Deng[1], Hui Tao[1], Yuling Qiu[2], Wenqiang Chen[2], Faquan Lin[1]

*Departments of [1]Clinical Laboratory, [2]Pediatric Laboratory, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China. *Equal contributors and co-first authors.*

Abstract: Objectives: We analyzed gene mutations in a Chinese family with hereditary spherocytosis (HS) combined with glucose-6-phosphate dehydrogenase (G6PD) deficiency, and evaluated differences in clinical features and diagnose the comorbidities. Methods: G6PD mutations were identified using PCR with fluorescence melting curve analysis. Erythrocyte membrane-protein defects were detected through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) experiments and Western blot analysis. Genetic mutations were analyzed using DNA sequencing. Results: Findings from PCR with fluorescence melting curve analysis revealed a 1388G>A mutation in the G6PD gene in the proband and his mother. The proband exhibited band 3 protein defects as determined by SDS-PAGE and western blot analysis. Gene sequencing results showed two missense mutations in exon 4 of the SLC4A1 gene; c.113A>C and c.166A>G. The same findings were also found in the proband's mother, but no mutation was detected in his father. Conclusions: Although the proband and his mother were both diagnosed as carriers of the same mutations, their degree of anemia were different. This suggests that in addition to gene mutations, environmental and other factors play an important role in the clinical manifestation of HS combined with G6PD deficiency. Findings from routine blood tests, morphological changes and gene sequencing are important for determining gene mutations all issues of concern. In addition, cell membrane analysis and a detailed family history should be performed to raise awareness of prenatal tests, diagnosis and treatment of such types of accompanied diseases.

Keywords: Hereditary spherocytosis, glucose-6-phosphate dehydrogenase deficiency, band 3, SLC4A1, hemolytic disease

Introduction

Hereditary spherocytosis (HS) is a chronic hemolytic disease associated with congenital defects in the erythrocyte membrane. The erythrocyte membrane is composed of α-spectrin, β-spectrin, ankyrin, band 3 and protein 4.2, which are encoded by *SPTA1, SPTB, ANK1, SLC4A1* and *EPB42,* respectively. In most cases HS is inherited in an autosomal dominant pattern. HS occurs at a frequency of 1 in 2000 individuals in Northern Europe and North America, and has been reported all over the world [1]. Symptoms of HS include anemia, jaundice and splenomegaly, while hematological features include increased peripheral spherocytosis and erythrocyte osmotic fragility. Splenectomy is an effective method for the treatment of HS and typically leads to improvements in anemia and jaundice. While splenectomy may reduce in the number of defective erythrocytes in the spleen, they remain present the peripheral blood.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common monogenetic diseases in the world, occurring in approximately 400 million people worldwide [2]. A higher incidence of G6PD deficiency is found in South China [3]. Clinical manifestations of G6PD vary greatly; while many people are asymptomatic, other exhibit symptoms including drug- or infection-induced hemolytic anemia, chronic non-spherical cell anemia and jaundice.

Clinically, HS combined with G6PD deficiency is rare and may be missed or prone to misdiagnosis. Here we report a case of mutations in both the *SLC4A1* and *G6PD* genes. We found that clinical differences may exist in carriers of the same two mutations.
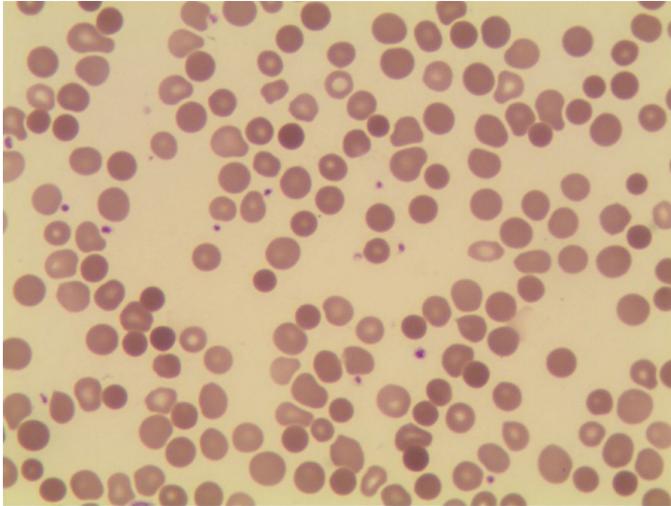
Figure 1. Peripheral blood smear showing erythrocyte morphology in the proband with hereditary spherocytosis.

Table 1. Laboratory test results from the proband and his family members

Item	Proband	Father	Mother	Normal range
Hb (g/dL)	70.5	152.5	135.3	120-160
RBC (× 10^{12}/L)	2.65	5.11	4.72	4-5.5
MCV (fl)	78.61	89.11	86.85	82-100
MCH (pg)	26.67	29.83	28.65	27-34
MSCV (fl)	66.26	87.25	85.23	84-104
MRV (fl)	87.46	101.08	101.48	101-119
Reticulocytes (10^9/L)	476	61	113	24-84
OF (fresh blood)	Increased	Normal	Normal	Normal
TBIL (µmol/L)	60.7	5.7	6.2	3.4-20.5
DBIL (µmol/L)	21.3	2.2	2.2	0-6.8
IBIL (µmol/L)	39.4	3.5	4	3.1-14.3
ALT (U/L)	12	37	15	9-60
AST (U/L)	23	28	22	15-45
G6PD (NBT)	1.38	13.8	13	6.8-20.5

Abbreviations: Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MSCV, mean sphered corpuscular volume; MRV, mean reticulocyte volume; TBIL, total bilirubin: DBIL, direct bilirubin; IBIL, Indirect bilirubin.

Materials and methods

Baseline data

Written informed consent was obtained from all participants in this study. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital at the Guangxi Medical University in China.

The proband of this study was a 5-year-old boy of Chinese Han ethnicity from Guangxi Zhuang Autonomous Region, China. He presented with pale complexion and experienced occasional brown urine over the past 5 years. He was diagnosed with G6PD deficiency. In September 2015, he was admitted to the hospital because of weakness and aggravated jaundice. Thalassemia was initially suspected because of biochemical results (total bilirubin: 59.5 µmol/L; direct bilirubin: 20.9 µmol/L; indirect bilirubin: 38.6 µmol/L; red blood cell count: 2.66 × 10^{12}/L, hemoglobin: 68 g/L, hematocrit: 20.9%). Five days later he was transferred to our hospital.

Upon physical examinations, the proband presented with an anemic appearance, scleral jaundice, superficial lymph nodes without swelling and normal heart and lung functions. The liver was not palpable, but the spleen was firm with clear margins, lacked tenderness and was located about 2.5 cm below the ribs. The proband had a history of G6PD deficiency, but no malaria and no acute intravascular hemolysis. Ultrasound results showed no abnormalities in the liver, gallbladder, bile duct, spleen, pancreas or kidney. Laboratory tests showed higher mean reticulocyte volume (MRV) than mean sphered corpuscular volume (MSCV), and increased erythrocyte osmotic fragility. Erythrocyte size was inconsistent and there was an approximate 15% increase in the number of spherocytes in blood smears (**Figure 1**). G6PD enzyme activity was reduced, but hemoglobin and thalassemia test results were normal and Coombs test result was negative. Based on his disease history, clinical symptoms and laboratory tests, the proband was diagnosed with HS. Blood and biochemical test results for his family members were normal, except for his older brother that presented with a moderate reduction in G6PD activity (**Table 1**). Gene mutation analysis for G6PD and HS was carried out in the proband and his parents.

Analysis of erythrocyte membrane proteins

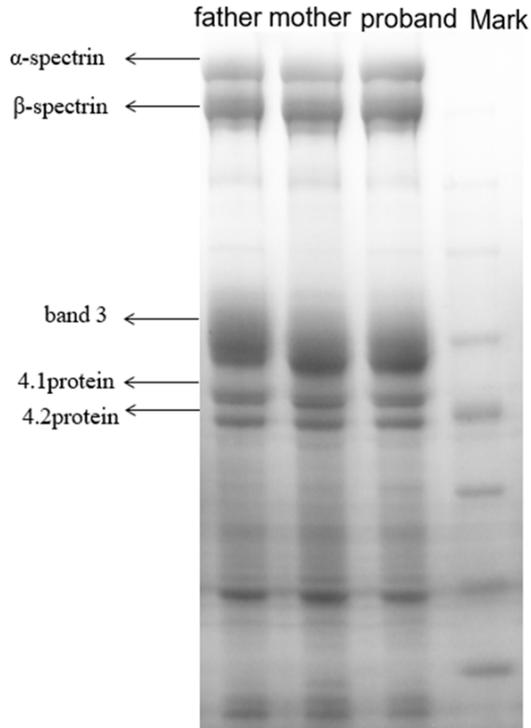Erythrocyte membrane proteins from the proband and his family members were subjected

Figure 2. SDS-PAGE analysis of erythrocyte membrane protein using Coomassie blue staining. Father (normal band 3 protein); Mother (partial band 3 protein defect); Proband (partial band 3 protein defect).

Figure 3. Western blot analysis of band 3 protein with β-actin as an internal reference. F, father (normal band 3 protein); M, mother (partial band 3 protein defect); P, proband (partial band 3 protein defect).

Table 2. Quantification of band 3 protein levels

Band 3/β-actin	Proband	Mother	Father
SDS-PAGE	4.93	5.80	7.21
Western blot	2.09	2.13	3.28

Abbreviation: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 3.5-17.0% linear gradient gel, and analyzed using Coomassie blue staining as reported previously [4, 5]. The gel was scanned using a Gel Doc 2000 gel imager and the band was quantitatively analyzed using the Quantity One analysis software (Bio-Rad, Singapore). Western blot analysis was performed using rabbit anti-band 3 protein monoclonal antibody, and then conjugated to goat anti-rabbit antibody with horseradish peroxidase. Beta-actin protein was used as an internal control. Rabbit anti-β-actin protein polyclonal antibody was used to quantitatively analyze the band 3 protein with Image J software.

Gene sequence analysis

A whole blood DNA extraction kit (QIAGEN, Hilden, Germany) was used to extract genomic DNA from the peripheral blood. Mutations in the G6PD gene were detected using a Glucose-6-Phosphate Dehydrogenase Gene Mutation Test Kit (Zeesan Biotech, Xiamen, China). Primers targeting to the sequences of exons and adjacent introns of SLC4A1 were designed us-
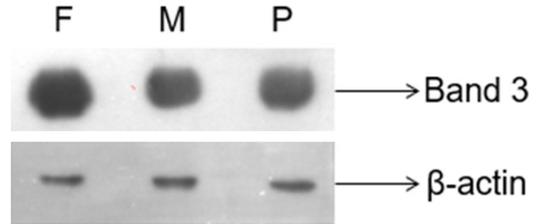
ing Primer 5 software, synthesized by the BGI (Shenzhen, China) and purified to >99% by PAGE. PCR reaction conditions were as follows: 35 cycles of denaturation at 95°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at the annealing temperature of each primer for 30 seconds, extension at 72°C for 40 seconds; followed by extension at 72°C for 8 minutes. The PCR product was stored at 4°C, purified and subjected to bi-directional DNA sequencing. The sequencing results were compared with standard sequences in the NCBI database to determine the mutation sites and variations in amino acid sequences.

Results

Laboratory test results

The routine blood tests and liver tests of the family members are shown in **Table 1**.

Analysis of membrane protein composition

SDS-PAGE electrophoresis (**Figure 2**) and western blot results (**Figure 3**) showed a significantly lighter band 3 protein in the proband and his mother than in his father. SDS-PAGE electrophoresis results were analyzed using Quantity One analysis software, and the relative quantification of band 3 protein was determined by the ratio of band 3/β-actin. The ratio of band 3/β-actin in the proband and his mother was lower than that in his father, indicating the pro-
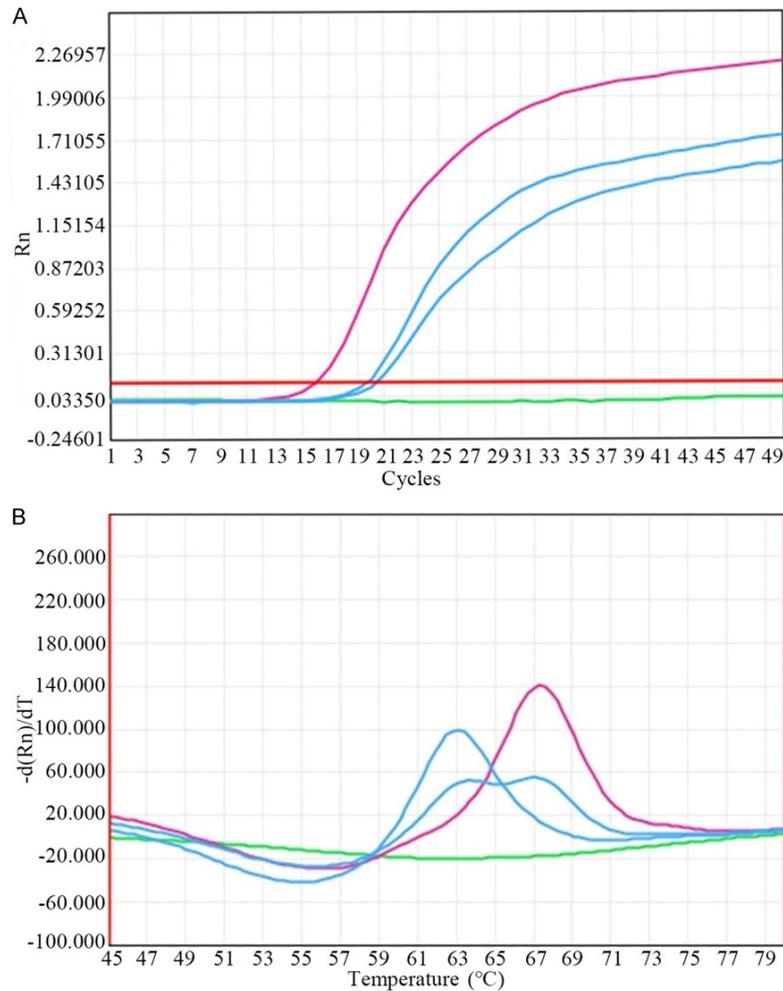
Figure 4. PCR-melting curve of the G6PD gene in the proband and his family members. Blue curve with single peak refers to the hemizygous mutation curve in the proband; blue curve with double peaks refers to the heterozygous mutation curve in the proband's mother; purple curve refers to positive control; and green curve refers to negative control.

band and his mother had partial defects in band 3 protein (**Table 2**). Quantitative analysis of western blot results using Image J software showed a significant reduction in the ratio of band 3/β-actin in the proband compared with his father (**Table 2**).

Gene analysis of G6PD and HS in the proband and his family members

The PCR-melting curve analysis showed the proband and his mother were diagnosed as hemizygous and heterozygous carriers of the G6PD gene 1388G>A mutation, respectively (**Figure 4**). DNA sequencing showed two missense mutations in exon 4 of the SLC4A1 gene in the proband: c.113A>C (Asp38→Ala38) and c.166A>G (Lys56→Glu56). These two muta-

tion sites were also verified in the proband's mother, but neither c.113A>C nor c.166A>G was found in his father (**Figure 5**).

Discussion

G6PD deficiency is a common enzyme deficiency disease in South China affecting 400 million people worldwide. Excessive erythrocyte damage and premature apoptosis leads to anemia in patients with G6PD deficiency. G6PD is a key enzyme in the pentose phosphate pathway. It produces the co-enzyme reduced nicotinamide adenine dinucleotide phosphate (NADPH) during the catalytic process to maintain glutathione (GSH) levels. Mitochondrial cells in the respiratory process produce free radicals and reactive oxygen species, both of which can damage DNA, lipoproteins and cell membranes leading to cell damage and lysis [6]. GSH is involved in the removal of reactive oxygen species during the respiratory process, protecting hemoglobin and the cell membrane thiol protein from oxidative damage [7]. Erythrocyte maturation depends on the antioxidant protection from GSH in the cytosolic pentose phosphate pathway. Consequently, NADP cannot be fully reduced to NADPH, when there is a G6PD deficiency in erythrocytes. Mixed disulfide comprising GSH and hemoglobin cannot be reduced and therefore accumulates in erythrocytes. These erythrocytes are then destroyed and phagocytosed in the spleen resulting in extravascular hemolysis, and the appearance of premature apoptosis, such as phosphatidylserine exposure, plasma membrane vesicle and cell shrinkage [8]. Studies have shown that G6PD deficiency also damages the physiological function of nucleated cells and accelerates cell senescence [9].
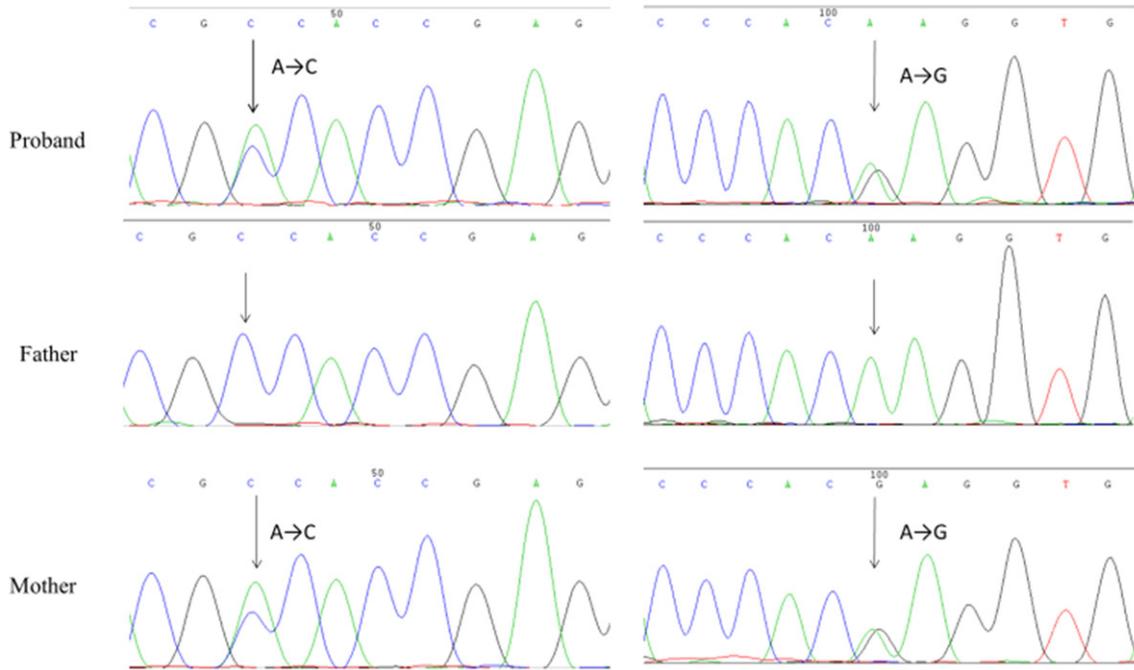
Figure 5. DNA sequencing results of exon 4 of the SLC4A1 gene. The proband and his mother were diagnosed as the carriers of two mutations in exon 4 of the SLC4A1 gene: c.113A>C (Asp38→Ala38) missense mutation and c.166A>G (Lys56→Glu56) missense mutation. Neither c.113A>C nor c.166A>G was found in the proband's father.

G6PD deficiency is inherited in an X-linked incomplete dominant pattern. Symptomatic patients are almost always males presenting with significantly reduced G6PD activity. Female heterozygote carriers often exhibit diversity in clinical manifestation; most are asymptomatic with normal G6PD activity, but some exhibit symptoms of chronic hemolytic anemia [10]. In this family, the PCR-melting curve of the *G6PD* gene showed the proband was a hemizygous carrier of a G6PD gene mutation and the mother was a carrier of a heterozygous mutation. The proband and his older brother both inherited the mother's mutated X chromosome. This explains why G6PD enzyme activity in the proband and his brother was moderately or severely reduced, but no obvious abnormalities occurred in his mother.

Chronic hemolysis is rare in patients with severe G6PD deficiency. Two possibilities should be considered when a G6PD deficient patient suffers from severe chronic hemolysis; one is a rare G6PD mutation and the second is the presence of another disease.

HS is a hereditary hemolytic disease characterized by spherical erythrocytosis in the peripheral blood. HS in inherited in an autosomal dominant pattern and occurs at an incidence of 1/2000 in northern Europe and North America [1]. The main clinical symptoms include anemia, jaundice and splenomegaly. The pathogenesis of HS is associated with defects in the erythrocyte resulting from genetic mutations in membrane proteins such as the band 3 protein, 4.2 protein, ankyrin, α-hemagglutinin and β-spectrin. The loss of membrane surface area leads to increased brittleness and decreased flexibility of the cell membrane. Consequently, the cells are more prone to rupture and are degraded by the spleen. The proband's mother was an asymptomatic carrier of HS. She had moderately elevated reticulocytes but no anemia, splenomegaly or hyperbilirubinemia. As such, she could only be diagnosed for HS by DNA sequencing.

HS combined with G6PD deficiency has rarely been reported in the literature [11-13]. Because the two different erythrocytes defects will interfere with the diagnosis of hemolysis, HS combined with G6PD deficiency is easily misdiagnosed or missed entirely. An erythrocyte osmotic fragility test is commonly used for the diagnosis of HS, with up to 95% sensitivity [10]. Of note, diagnosis based on the erythrocyte osmotic fragility can give a false negative result in

the presence of reticulocytes, thalassemia and iron deficiency. G6PD enzyme activity is also susceptible to reticulocyte interference. Therefore, if G6PD activity is normal when the number of reticulocytes is increased, gene sequencing should be used for the diagnosis of G6PD deficiency.

The molecular pathological mechanism underlying HS in proband is likely attributed to the synergistic effects of the c.113A>C (Asp38Ala) and c.166A>G (Lys56Glu) in exon 4 of SLC4A1, combined with the c.1388G>A (Arg463His) of G6PD gene.

Arg463His was the most common mutation that has been known as a cause for G6PD deficiency. Asp38Ala was previously reported as a polymorphism. For example, Eber et al. [14, 15] reported it to be a low-frequency ($P = 0.01$, n = 100) polymorphic mutation of band 3. Yawata et al. [16] reported it to be a very common polymorphism that may also be causative of HS. Lys56Glu was also a polymorphism which was characterized by slower migration than the normal band 3 on SDS-PAGE [17], and reports have shown that it could cause HS [18]. In our study, only these two mutations related to HS were identified in proband and his mother, but they were not detected in other family members including brother and his father. Therefore, it appears to be a pathological mutation that causes a band 3 defect in proband. This differs from the findings of studies conducted outside China in different ethnic groups and/or even within the same ethnic group, in which a band 3 deficiency is caused by different SLC4A1 mutations [19].

Eosin-5'-maleimide (EMA) binding tests and acidified glycerol hemolysis tests are considered highly effective diagnostic indicators of HS [20, 21], with a sensitivity of 92.7% and 95%, respectively. Kedar et al. [12] reported a case of HS with spectrin and G6PD deficiency in which skelemin abnormalities in the erythrocyte membrane could be detected by acidified glycerol experiments and erythrocyte osmotic fragility test, but were not observed by SDS-PAGE electrophoresis. This patient presented with an obvious tendency for hemolysis. The patient inherited HS from their father and G6PD deficiency from their mother; however neither of the parents showed any obvious clinical symptoms. Jamwal et al. [13] also reported a

case of missed diagnosis of HS. This patient had been diagnosed initially with G6PD deficiency, but multiple blood transfusions and use of oxidative drugs did not improve recurrent anemia and jaundice. Subsequently, this patient was diagnosed with HS based on EMA fluorescence labeling and erythrocyte osmotic fragility tests.

As excessive reticulocytes can affect the concentration gradient of gel separation for abnormal cell membrane proteins [22], it is important to note that approximately 10% of HS patients cannot be diagnosed by SDS-PAGE electrophoresis. Blood tests such as MSCV and MCV are highly effective diagnostic indicators for HS. Mean corpuscular hemoglobin concentration (MCHC) is used as one of the diagnostic indicators of HS in England [1]. However, it exhibits imprecise sensitivity and specificity to HS; when MCHC>34.7 g/l, the sensitivity to HS is 73.3% and the specificity is 72.6% [9]. In addition, increased bilirubin in the blood will interfere with the detection of MCHC, resulting in a false positive result. A study from Broséus et al. [23] showed that the sensitivity and specificity to HSV was 100% and 90.57%, respectively, when MCV-MSCV>9.6 fl. However, in our studies [24] we found that only 77.19% of HS patients exhibited MCV-MSCV>9.6 fl in routine blood examinations. However, when MSCV<MCV, the sensitivity and specificity were increased to 98.25% and 99.10%, respectively. In addition, we compared the hemolytic parameters of four causes of anemia, including HS, thalassemia, autoimmune hemolytic anemia and G6PD deficiency. We found significantly lowered MRV in the HS group compared with the normal control, thalassemia, autoimmune hemolytic anemia and G6PD deficiency groups. When the MRV≤95.77 fl, HS sensitivity and specificity were 86.80% and 91.20%, respectively [25]. In this study, the proband exhibited MCV-MSCV = 12.35 fl and MRV = 87.46 fl, values that are different from patients with only a G6PD deficiency.

In this case report, the proband also appeared to have two hemolytic diseases in different hereditary patterns; the gene-encoding band 3 protein was located on chromosome 17q21, and the *G6PD* deficiency gene was located on the X chromosome q28, with no linkage relationship. Onset of HS combined with G6PD deficiency occurs at random. Although gene sequencing revealed the same mutation sites in the

proband and his mother, the proband's mother had no clinical signs while the proband presented with moderate hemolysis, reduced hemoglobin, elevated reticulocytes, jaundice and splenomegaly. These findings demonstrate that the same genetic mutation in different individuals may present with different clinical manifestations. This suggests a complex pathogenic mechanism by which *G6PD* and *SLC4A1* gene mutations cause hemolysis. HS combined with G6PD deficiency increased the tendency of hemolysis in the proband. Likely, the partial deficiency of band 3 protein makes erythrocyte membrane proteins more susceptible to oxidative damage. The G6PD deficiency results in oxidative stress that further damages the connexin protein of band 3 protein. The connexin protein includes protein 4.2 and ankyrin, through which band 3 attaches to β-spectrin and the cytoskeleton. This negative cycle ultimately leads to the loss of band 3 protein and more severe clinical symptoms. The normal level of G6PD in the proband's mother, however, may prevent the loss of cytoskeleton band 3 protein.

HS mutations are diverse, and band 3 protein mutations have been increasingly reported in studies. Bolton-Maggs *et al.* [1] showed that mutations in band 3 were identified in 15% to 20% of HS cases that included missense, nonsense and larger mutant proteins. Mariani *et al.* [26] showed the data from 300 consecutive patients with hereditary spherocytosis; band 3 protein was deficient in 54% of those cases. G6PD deficiency has a higher incidence in Southeast Asia and in the Mediterranean coastal region, but little is reported on chronic hemolysis in G6PD deficiency patients. However, individuals carrying *G6PD* mutated genes can still suffer from oxidative stress-induced hemolysis [27]. In this study, the proband had two chronic hereditary diseases with different pathogeneses, and further studies on the relationship between these two diseases are still required. Of note, if abnormal anemia and jaundice occur in a G6PD deficiency patient, clinicians should consider other blood diseases in the patient and perform relevant examinations accordingly. In an oxidative environment, G6PD-deficient erythrocytes are unable to maintain the reduced state of GSH, which may affect the integrity of the cell membrane. Additionally, if there is another factor related to membrane damage,

a synergistic effect on the cell surface will shorten the life of erythrocytes. Further exploration on whether this synergistic effect only occurs on the erythrocyte membrane in patients with G6PD deficiency combined with band 3 protein defect is needed.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Faquan Lin, Department of Clinical Laboratory, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China. Tel: +86-771-5329287; E-mail: fqlin1998@163.com

References

[1]   Bolton-Maggs PH, Langer JC, Iolascon A, Tittensor P, King MJ. Guidelines for the diagnosis and management of hereditary spherocytosis--2011 update. Br J Haematol 2012; 156: 37-49.

[2]   Nkhoma ET, Poole C, Vannappagari V, Hall SA, Beutler E. The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. Blood Cells Mol Dis 2009; 42: 267-278.

[3]   Liu WL, Li F, He ZX, Jiang HY, Ai R. Glucose-6-phosphate dehydrogenase qingzhen: identification of a novel splice mutation (IVS5-1 G>A). Pediatr Blood Cancer 2012; 58: 825-826.

[4]   Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680-685.

[5]   Fairbanks G, Steck TL, Wallach DF. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 1971; 10: 2606-2617.

[6]   Jalil N, Azma RZ, Mohamed E, Ithnin A, Alauddin H, Baya SN, Othman A. Evaluation of glucose-6-phosphate dehydrogenase stability in stored blood samples. EXCLI J 2016; 15: 155-162.

[7]   Luzzatto L, Nannelli C, Notaro R. Glucose-6-phosphate dehydrogenase deficiency. Hematol Oncol Clin North Am 2016; 30: 373-393.

[8]   Lang E, Qadri SM, Lang F. Killing me softly-suicidal erythrocyte death. Int J Biochem Cell Biol 2012; 44: 1236-1243.

[9]   Lee JW, Choi AH, Ham M, Kim JW, Choe SS, Park J, Lee GY, Yoon KH, Kim JB. G6PD up-regulation promotes pancreatic beta-cell dysfunction. Endocrinology 2011; 152: 793-803.

[10]   Minucci A, Moradkhani K, Hwang MJ, Zuppi C, Giardina B, Capoluongo E. Glucose-6-phosphate dehydrogenase (G6PD) mutations database: review of the "old" and update of the new mutations. Blood Cells Mol Dis 2012; 48: 154-165.

[11]   Kilinc Y, Zorludemir U, Olcay I. Gallstones due to hereditary spherocytosis with glucose-6-phosphate dehydrogenase deficiency. Turk J Pediatr 1987; 29: 121-126.

[12]   Kedar PS, Colah RB, Ghosh K, Mohanty D. Hereditary spherocytosis in association with severe G6PD deficiency: report of an unusual case. Clin Chim Acta 2004; 344: 221-224.

[13]   Jamwal M, Aggarwal A, Kumar V, Sharma P, Sachdeva MU, Bansal D, Malhotra P, Das R. Disease-modifying influences of coexistent g6pd-deficiency, gilbert syndrome and deletional alpha thalassemia in hereditary spherocytosis: a report of three cases. Clin Chim Acta 2016; 458: 51-4.

[14]   Eber SW, Gonzalez JM, Lux ML, Scarpa AL, Tse WT, Dornwell M, Herbers J, Kugler W, Ozcan R, Pekrun A, Gallagher PG, Schroter W, Forget BG, Lux SE. Ankyrin-1 mutations are a major cause of dominant and recessive hereditary spherocytosis. Nat Genet 1996; 13: 214-218.

[15]   Kanzaki A, Hayette S, Morle L, Inoue F, Matsuyama R, Inoue T, Yawata A, Wada H, Vallier A, Alloisio N, Yawata Y, Delaunay J. Total absence of protein 4.2 and partial deficiency of band 3 in hereditary spherocytosis. Br J Haematol 1997; 99: 522-530.

[16]   Yawata Y, Kanzaki A, Yawata A, Doerfler W, Ozcan R, Eber SW. Characteristic features of the genotype and phenotype of hereditary spherocytosis in the Japanese population. Int J Hematol 2000; 71: 118-135.

[17]   Spring FA, Bruce LJ, Anstee DJ, Tanner MJ. A red cell band 3 variant with altered stilbene disulphonate binding is associated with the Diego (Dia) blood group antigen. Biochem J 1992; 288: 713-716.

[18]   Sanchez-Lopez JY, Camacho-Torres AL, Ibarra B, Tintos JA, Perea FJ. Analysis of the SLC4A1 gene in three Mexican patients with hereditary spherocytosis: report of a novel mutation. Genet Mol Biol 2010; 33: 9-11.

[19]   Bouhassira EE, Schwartz RS, Yawata Y, Ata K, Kanzaki A, Qiu JJ, Nagel RL, Rybicki AC. An alanine-to-threonine substitution in protein 4.2 cDNA is associated with a Japanese form of hereditary hemolytic anemia (protein 4.2NIPPON). Blood 1992; 79: 1846-1854.

[20]   Bianchi P, Fermo E, Vercellati C, Marcello AP, Porretti L, Cortelezzi A, Barcellini W, Zanella A. Diagnostic power of laboratory tests for hereditary spherocytosis: a comparison study in 150 patients grouped according to molecular and clinical characteristics. Haematologica 2012; 97: 516-523.

[21]   Hunt L, Greenwood D, Heimpel H, Noel N, Whiteway A, King MJ. Toward the harmonization of result presentation for the eosin-5'-maleimide binding test in the diagnosis of hereditary spherocytosis. Cytometry B Clin Cytom 2015; 88: 50-57.

[22]   King MJ, Zanella A. Hereditary red cell membrane disorders and laboratory diagnostic testing. Int J Lab Hematol 2013; 35: 237-243.

[23]   Broseus J, Visomblain B, Guy J, Maynadie M, Girodon F. Evaluation of mean sphered corpuscular volume for predicting hereditary spherocytosis. Int J Lab Hematol 2010; 32: 519-523.

[24]   Liao L, Deng ZF, Qiu YL, Chen P, Chen WQ, Lin FQ. Values of mean cell volume and mean sphered cell volume can differentiate hereditary spherocytosis and thalassemia. Hematology 2014; 19: 393-396.

[25]   Xu Y, Yang W, Liao L, Deng Z, Qiu Y, Chen W, Lin F. Mean reticulocyte volume: a specific parameter to screen for hereditary spherocytosis. Eur J Haematol 2016; 96: 170-174.

[26]   Mariani M, Barcellini W, Vercellati C, Marcello AP, Fermo E, Pedotti P, Boschetti C, Zanella A. Clinical and hematologic features of 300 patients affected by hereditary spherocytosis grouped according to the type of the membrane protein defect. Haematologica 2008; 93: 1310-1317.

[27]   Sukumar S, Colah R, Mohanty D. G6PD gene mutations in India producing drug-induced haemolytic anaemia. Br J Haematol 2002; 116: 671-672.