Clin. Lab. 2025;71:XXX-XXX

CASE REPORT

Pyrimidine-5′-Nucleotidase Deficiency: a New Homozygous NT5C3A Mutation (c.693+1G>A variant)

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SUMMARY

Background: **Erythrocytes have an average lifespan of 120 days, after which they are typically removed by macrophages in the reticuloendothelial system. Hemolytic anemia can shorten erythrocyte lifespan, leading to varying clinical presentations depending on whether hemolysis occurs intravascularly or extravascularly. Among intrinsic causes of hemolysis, pyrimidine 5'-nucleotidase (P5N) deficiency is a notable condition, often presenting as non-spherocytic hemolytic anemia.**
Methods: **We report a case of a 65-year-old female patient with systemic lupus erythematosus and a history of splenectomy, who was admitted for evaluation of persistent hemolytic crises. Clinical examination, peripheral blood smear analysis, and genetic testing were performed, including next-generation sequencing to identify mutations in the NT5C3A gene associated with P5N deficiency.**
Results: **The patient exhibited macrocytic anemia and basophilic stippling on peripheral blood smear, with normal results from osmotic fragility tests and G6PD levels. Genetic testing revealed a homozygous c.693+1G>A variant in the NT5C3A gene, classified as possibly pathogenic based on ACMG criteria. This variant is linked to P5N deficiency, which aligns with the patient's clinical presentation of non-immune hemolytic anemia.**
Conclusions: **The identification of the NT5C3A gene mutation through next-generation sequencing highlights the significance of molecular technologies in diagnosing rare forms of hemolytic anemia. This case underscores the necessity for genetic counseling for affected individuals and their families, as well as the importance of continued follow-up and supportive care in managing hemolytic anemia related to enzyme deficiencies.**
(Clin. Lab. 2025;71:xx-xx. DOI: 10.7754/Clin.Lab.2024.240807)

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Case Report accepted September 2, 2024

KEYWORDS

hemolytic anemia, pyrimidine 5'-nucleotidase deficiency, NT5C3A gene

INTRODUCTION

The average lifespan of erythrocytes is 120 days, at which point their life is ended by macrophages belonging to the reticuloendothelial system found in the bone marrow, spleen, or liver. Alternatively, erythrocyte lifespan can be shortened due to internal or external causes, a condition referred to as hemolytic anemia. The clinical and laboratory findings may vary depending on whether hemolysis occurs intravascularly or extravascularly [1]. When discussing intrinsic causes of erythro-

cyte hemolysis, we can categorize them into membrane defects, metabolic disorders arising from enzyme deficiencies, and defects in hemoglobin synthesis. A mature erythrocyte is capable of efficiently transporting oxygen to tissues the moment it leaves the bone marrow. The biconcave shape of the erythrocyte creates an ideal environment for gas exchange. However, maintaining this specialized biconcave shape is possible only by losing the nucleus and organelles [2]. Because erythrocytes lack a nucleus, mitochondria, and ribosomes, they do not have the capacity for oxidative phosphorylation or protein synthesis. Nonetheless, erythrocytes must maintain an active metabolism to preserve membrane integrity and cell flexibility and to keep hemoglobin in the necessary functional reduced form for oxygen distribution. The primary energy source for erythrocytes is adenosine triphosphate (ATP) obtained through anaerobic glycolysis [3]. Purine metabolism meets the ATP and guanosine triphosphate (GTP) needs of erythrocytes, while pyrimidine derivatives are present in very small amounts as they are depleted during maturation. The most important enzymes in nucleotide metabolism include pyrimidine 5'-nucleotidase (P5N), which is involved in pyrimidine metabolism, and adenylate kinase and adenosine deaminase (ADA), which are involved in purine metabolism. A deficiency of any of these three enzymes can lead to hereditary non-spherocytic hemolytic anemia [4]. P5N deficiency is the third most common cause of non-spherocytic hereditary hemolytic anemia worldwide, following G6PD and pyruvate kinase deficiencies. Its inheritance is autosomal recessive, typically presenting with mild to moderate hemolytic anemia, splenomegaly, and jaundice. Since P5N removes pyrimidine nucleotides from erythrocytes, a deficiency of this enzyme leads to the accumulation of pyrimidine derivatives. Increased pyrimidine nucleotides manifest as basophilic stippling in peripheral smears due to aggregate formation [5]. Here, we present a rare case associated with P5N deficiency, diagnosed through next-generation sequencing in a patient exhibiting features of non-spherocytic hemolytic anemia.

CASE PRESENTATION

A 65-year-old female patient has a known diagnosis of systemic lupus erythematosus for seven years and a history of papillary thyroid cancer treated seven years ago. The patient has a history of splenectomy due to splenomegaly 20 years ago, but detailed medical documentation regarding the reason for the splenectomy could not be obtained. The patient has a history of immune hemolytic anemia and immune thrombocytopenia associated with systemic lupus erythematosus, for which she has received steroid treatment, intravenous immunoglobulin G, and 5 courses of rituximab. Before receiving rituximab, both direct and indirect Coombs tests were positive; after treatment, both tests returned negative results. Nevertheless, the patient continues to experience hemolytic crises and was admitted to our unit for further evaluation. During the physical examination, the patient exhibited pallor and jaundice. There was no organomegaly. The peripheral blood smear showed macrocytosis, polychromasia, and basophilic stippling. The osmotic fragility test was normal. Glucose-6-phosphate dehydrogenase (G6PD) levels were within normal limits. Hemoglobin electrophoresis was normal. Bone marrow aspiration revealed hypercellularity with an increase in erythroid series, and no sideroblasts were detected. Laboratory tests indicated normal levels of folic acid and vitamin B12. Liver and kidney function tests were normal. The complete blood count showed leukocytes at 7,800/mm^3, platelets at 475 x 10^9/L, hemoglobin at 8.8 g/dL, hematocrit at 28.1%, MCV at 128.3 fL, red blood cell count at 2,190,000/mm^3, reticulocytes at 4%, total bilirubin at 3.23 mg/dL, direct bilirubin at 0.87 mg/dL, lactate dehydrogenase at 393 IU, and haptoglobin greater than 10 mg/dL. Urinary urobilinogen was normal. Tests for paroxysmal nocturnal hemoglobinuria were negative for the PNH clone. Genetic testing has been planned for the patient. As a result of the new generation gene sequencing performed, a homozygous c.693+1G>A variant was identified in the NT5C3A gene. According to the classification by the American College of Medical Genetics, this finding is classified as PVS1 (which may lead to RNA degradation, potentially inhibiting protein formation and function) and PM2 (not observed in population databases or extremely rare), thereby being classified as possibly pathogenic. The identified variant in the NT5C3A gene has been associated with pyrimidine 5'-nucleotidase (P5N) deficiency in the literature, which was consistent with the clinical and laboratory findings in our case. The patient's ongoing non-immune hemolytic crises were attributed to this variant in the genetic region. It was recommended that the patient receive genetic counseling and that family members be referred to the genetic clinic for genetic screening. Additionally, the patient was provided with folic acid support and followed up at the hematology outpatient clinic.

DISCUSSION

The NT5C3A gene encodes a member of the 5'-nucleotidase enzyme family that catalyzes the phosphorylation of 5'-nucleoside monophosphates. The protein encoded by this gene is the type 1 isoenzyme of pyrimidine 5'-nucleotidase, responsible for catalyzing the phosphorylation of pyrimidine 5' monophosphates. Mutations in this gene are a cause of hemolytic anemia due to deficiency of uridine 5'-monophosphate hydrolase. Alternative splicing variants coding multiple isoforms for this gene have been observed [6]. In pyrimidine 5'-nucleotidase deficiency, which is an autosomal recessive condition, loss of NT5C3 can lead to the accumulation of pyrimidine nucleotides in high concentrations in erythrocytes. This deficiency has been associated with

moderate hemolytic anemia, jaundice, splenomegaly, and prominent basophilic stippling [7]. Advances in molecular technologies, particularly next-generation sequencing, inspire first-line applications for identifying potential mutations and determining new causal genes in patients showing a rare form of hemolytic anemia. Twenty different mutations in the P5N-1 gene have been identified in 37 patients from 30 families affected by P5N deficiency. Mutations are missense mutations, in-frame amino acid deletions, nonsense mutations, deletions, additions or changes [5]. Although transcriptomic and functional studies have shown that the NT5C3A gene is expressed in various tissues and cell lines, only one inherited disease (P5ND) has been linked to mutations in this gene [8].

CONCLUSION

Advances in molecular technologies are inspiring us in first-line applications, especially for next-generation sequencing to drive potential mutations and arrive at the latest causative genes that demonstrate a rare formula of hemolytic anemia.

Patient Consent:
Written informed consent was obtained from the patient for publication of this case report.

Declaration of Interest:
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. No conflict of interest was declared by the authors.

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