Dehydrated hereditary stomatocytosis with new missense mutations in PIEZO1 through the use of next-generation sequencing panel

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Abstract

In this case study, we report an 11-year-old male patient who had jaundice, hepatosplenomegaly, and chronic mild congenital non-autoimmune hemolytic anemia. In our patient, a novel homozygous missense mutation in the PIEZO1 gene was detected using a gene-targeted Next-Generation Sequencing panel: c.3364G>A (p.Glu1122Lys), confirming the diagnosis of DHS.

Introduction

Dehydrated hereditary stomatocytosis (DHS) is one of the non-immune hemolytic anemias presenting with broad-spectrum clinical findings due to increased erythrocyte membrane cation permeability. DHS The estimated prevalence was reported at 1/8000 and 6-fold greater than prior estimates.[1] Although the clinical findings of DHS vary widely, all forms of DHS have hemolysis and anemia that vary from mild to severe are present. Also, it can present with jaundice, pallor, weakness, splenomegaly and gallstones as in all hemolytic anemias. This disease is divided into two groups: either isolated erythroid phenotype (non-syndromic) or extra-hematological manifestations (syndromic). The syndromic form is examined in three subtypes. These are classified as follows: (i) Stomatin deficient cryohydrocytosis with mental retardation, seizures and hepatosplenomegaly, (ii) Phytosterolemia nonleaky stomatocytosis with macrothrombocytopenia, (iii) DHS with perinatal edema and/or pseudohyperkalemia. Nonsyndromic forms are classified as: (i) Overhydrated hereditary stomatocytosis, (ii) Cryohydrocytosis, (iii) DHS and (iv)Familial pseudohyperkalemia. Mutations in ABCG5, ABCG8, PIEZO1, SLC2A1, ABCB6, KCNN4 and RHAG genes are responsible for all subtypes of that disease.[1] In recent years, studies have shown that PIEZO1 mutations are caused by both syndromic and nonsyndromic forms of DHS: Type 1a and Type 1b.[1] Here, we want to present a case of hereditary dehydrated stomatocytosis diagnosed at 11 years old due to the detection of a new homozygous missense mutation (c.3364G> A (p.Glu1122Lys) in the PIEZO1 gene with a Next-Generation Sequencing (NGS) panel, who has been followed up for non-immune hemolytic anemia since the age of one.

Case presentation

An 11 years old male patient has been followed up due to chronic non-immune, non-spherocytic hemolytic anemia of unknown origin since one year old. He was born by C/S as a term baby and second pregnancy. His parents were consanguineous. His one brother has been following up due to an unknown cause of hemolytic anemia. On the examination, he had jaundice, pallor appearance, and 3 cm splenomegaly, and 2 cm hepatomegaly. At the last laboratory results, his blood count was hemoglobin 9.0 gr/dl, mean

corpuscular volume 80 fL, mean corpuscular hemoglobin 23 pg, mean corpuscular hemoglobin concentration (MCHC) 33 g/dL, red cell distribution width 17.7 % (n=11-14 %), platelet 255000/mm^3, leukocyte 6200/mm^3, absolute neutrophil count 4400/mm^3, absolute reticulocyte number 210.0 x10^3/μL. The direct antiglobulin test was negative. Levels of hepatic enzymes were unconjugated bilirubin 2.1 mg/dl, conjugated bilirubin 0.7 mg/dl, lactate dehydrogenase 806 IU/L (n=180-430 IU/L). Folic acid and vitamin B12 levels were normal. Haptoglobin was below 30 mg/dl. Ferritin level was 450 ng/mL. His peripheral blood smear revealed severe polychromasia, anisocytosis, tear cells, a few fragmented erythrocytes, target cells, 5 % stomatocytes, increased normoblasts, and Howell-Jolly body in the erythrocyte. He presented with mild anemia, not needing frequent erythrocyte transfusions, jaundice, hepatosplenomegaly, high levels of unconjugated bilirubin and low levels of haptoglobin. Hemoglobin electrophoresis was normal. Cryohemolysis and osmotic fragility test were normal, as also CD 55 and CD 59 expression by flow cytometry. The flow cytometric EMA-binding test (expressed as % of decrease in fluorescence) was detected 10 % (normal values 11 %). Due to difficulty in reaching a diagnosis, the patient's blood sample was tested in collaboration with Fondazione Ospedale Maggiore Policlinico(Milan, Italy). The activity of the most common red cell enzymes (glucose-6-phosphate dehydrogenase, pyruvate kinase, hexokinase, glucose-6-phosphate isomerase, phosphofructokinase, glyceraldehyde-P-dehydrogenase, phosphoglycerate kinase, 6-phosphogluconate dehydrogenase, adenylate kinase, purine/pyrimidine ratio (to exclude the presence of pyrimidine 5' nucleotidase deficiency) was normal. After receiving the patient's informed consent, the DNA sample of the patient was analysed on an NGS targeted panel SureDesign software (Agilent), containing 40 genes associated with congenital hemolytic anemia. Libraries were obtained by HaloPlexHS Target Enrichment System Kit and sequenced on a MiSeq platform (Illumina).[2] In our patient, new missense mutations (c.3364G> A, p.Glu1122Lys) were identified in PIEZO1 associated with DHS. Mutations were confirmed by the Sanger method, as previously reported. Written informed consent was obtained from the patient and parents.

Discussion

DHS is characterized by alteration of the red blood cell membrane permeability related to alterations of the intracellular cationic content and cell volume.[3] Generally, DHS is a group of well-compensated hemolytic anemia diseases manifested by high reticulocyte count, macrocytosis, and mild jaundice.[4]Because 5 % stomatocytes was seen in our patient's peripheral smear, hereditary stomatocytosis was not considered initially and other causes of non-immune hemolytic anemia were investigated. Since stomatocytes are rare in the peripheral smears, it is challenging to make the definitive diagnosis of DHS, just like our patient.

DHS is inherited as an autosomal dominant trait. The gene locus was first localized at 16q23–24.[5] Several years later, PIEZO1, a mechanosensitive cation channel, was isolated by exome sequencing in the DHS patients and realized that it could cause both syndromic and nonsyndromic DHS. [1,6] The identified mutations are mostly missense and mainly located in the highly conserved C-terminus of the protein. The mutations cause delayed inactivation of the channel and increased cation permeability that leads to erythrocyte dehydration.[7] Recently, a novel gene, KCNN4, has been identified as causative of the second form of the DHS, named DHS2, in six different families.[8] However, 83 % of DHS patients carried PIEZO1 mutations. A new homozygous missense mutation (c.3364G> A, p.Glu1122Lys) was detected in our patient's PIEZO1 gene, which is the most common one, using the Whole NGS target sequencing method. Since our patient did not have a history of perinatal edema or hydrops fetalis, it was accepted as nonsyndromic DHS.

Although it has shown that these two forms of DHS have very similar phenotypes, there are also some differences. Especially, while the hemoglobin values and MCV values of DHS patients with KCNN4 mutation were lower, the reticulocyte count was higher. Chronic and mild hemolytic anemia has been shown more frequently in patients with PIEZO1 mutation.[8] Our patient was diagnosed at 11 years. Hemoglobin value was 9 g/dl. In the study of Andolfo et al., they reported that the age of onset of symptoms was 13.7 ± 2.2 years and the age of diagnosis was 36.3 ± 3.0 years in patients with PIEZO1 mutation. The onset of symptoms in our case was similar to the findings obtained in previous studies. However, because of studying genetic tests with NGS, the mutation was determined and a definite diagnosis could be made at a much earlier age. Unlike other DHS, there are two significant points in clinical follow-up in DHS: transfusion-independent

iron accumulation, especially in the liver and the contraindication of splenectomy. For these reasons, early diagnosis at the onset of symptoms is important for the subsequent clinical management of the patient. In recent years, it has been shown that senicapoc treatment can be effective in DHS patients.[9] Thanks to its early diagnosis, it may also allow the use of new treatment methods that are on the agenda in the treatment of the DHS before complications develop.

In conclusion, we here present a new case of DHS diagnosed through the aim of interlaboratory collaboration and the use of advanced technologies as NGS targeted sequencing platforms. Rare anemia can be challenging to diagnose in a developing country due to limited resources, financial constraints, and the disease's rarity. Expert laboratories are needed for an easier, faster, and more accurate diagnosis.

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Running title: Dehydrated hereditary stomatocytosis

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