Diagnosing dehydrated hereditary stomatocytosis due to a KCNN4 Gardos channel mutation: understanding challenges through study of a multi-generational family

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To the Editor,

Dehydrated Hereditary Stomatocytosis (DHSt) is autosomal dominant hereditary anemia with an estimated incidence of 1:50,000 births. DHSt is characterized by largely compensated hemolysis and splenomegaly and is attributed primarily to mutations in either the PIEZO1 or KCNN4 genes [1]. The majority of described DHSt cases result from gain-of-function mutations in the mechanosensitive cation channel gene PIEZO1, leading to an inappropriate increase in calcium influx. This, in turn, activates the calcium-sensitive, potassium-selective Gardos channel which mediates potassium conductance and regulates red cell volume [2]. Less frequently, gain-of-function mutations have been identified in the Gardos channel gene, KCNN4, that alter calcium sensitivity and result in a more active channel. Therefore, DHSt due to KCNN4 mutation is aptly termed a Gardos channelopathy [2–4]. Though a complex compensatory mechanism has been proposed in those with KCNN4 mutations, the increased cation leak across the red blood cell membrane in cases with either PIEZO1 or KCNN4 mutations is accompanied by intracellular dehydration and the formation of stomatocytes [4].

Since the first reports in 2015, to our knowledge, KCNN4 mutations have been identified in ten DHSt families [2–6]. PIEZO1 mutations in DHSt are more prevalent, with at least eight times as many cases identified [2]. Here we report the eleventh family with DHSt due to a KCNN4 substitution mutation that results in p.R352H. This family highlights the barriers to diagnosis, which include the variable phenotype, paucity of characteristic xerocytes and stomatocytes on peripheral smear, and omission of KCNN4 on many commercially available hemolytic anemia panels. The correct diagnosis was made only after in-house whole-exome sequencing was performed. Recognition and appropriate diagnosis of this disease are critical to better understand the true prevalence and clinical phenotype of individuals carrying KCNN4 mutations and to facilitate the development of new treatments.

Five subjects, from a single US Caucasian family of European descent with no extended history of jaundice or anemia, were enrolled in this study after International Review Board (IRB) approval and informed consent. The subjects included the proband, her unaffected husband, her two children (both affected), and an unaffected grandchild. The proband was jaundiced, with laboratory studies consistent with hemolytic anemia at birth (Table 1). She had normal growth and development, but underwent a splenectomy at 3 years of age. Therefore, DHSt due to KCNN4 mutation is aptly termed a Gardos channelopathy [2–4]. Though a complex compensatory mechanism has been proposed in those with KCNN4 mutations, the increased cation leak across the red blood cell membrane in cases with either PIEZO1 or KCNN4 mutations is accompanied by intracellular dehydration and the formation of stomatocytes [4].

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### TABLE 1  Hematologic data for proband, son, and daughter.

|       | Age  | Hgb (13–17 g/dl) | MCV (81–96 fl) | MCHC (32–36 g/dl) | RDW (11.5–14.2%) | Retic count (0.4–2%) | Platelet count (150–350 K/µl) | Total bilirubin (0–1.2 mg/dl) | Ferritin F (13–150 ng/ml) | LDH (100–250 M (30–400 ng/ml) |
|-------|------|-----------------|----------------|-----------------|-----------------|----------------------|-------------------------------|----------------------------|-------------------------|--------------------------|
| Proband | 61   | 10.5 ±0.3       | 106.5 ±1.3     | 34.1 ±0.5       | 15 ±0.4         | 12.6 ±2              | 532.2 ±32.9                   | 6 ±0.9 (Bili D < 0.1 - 0.6) | 503.2 ±113.2            | 438.5 ±153               |
| Daughter | 39   | 11.1 ±0.7       | 99.9 ±2.9      | 33.9 ±1.0       | 16.1 ±0.3       | 6.7 ±1.5             | 192.3 ±49.4                   | 2.3 ±0.5 (Bili D < 0.1-0.3) | 2225 ±84.5              | 301 ±148                |
| Son     | 34   | 9.3 ±0.4        | 98.8 ±3.1      | 32.9 ±0.7       | 21.2 ±0.6       | >22                  | 837 ±64.7                     | 5.7 ±0.7 (Bili D < 0.1 - 0.4) | 1061.9 ±53.5            | 483.5 ±233.5            |

*Means calculated from at least 25 measurements, except for Ferritin and LDH which are means of at least four values.

**Splenectomized.

*Post-menopausal. Prior to menopause, her average ferritin was 369 ng/ml (±109.8).

**Ferritin values prior to iron chelation therapy.

Peripheral smear showed anisocytosis with hypochromia and a few stomatocytes, occasional Howell-Jolly bodies, and stippling. A targeted Next Generation Hereditary Hemolytic Anemia sequencing panel performed on the proband by an outside reference laboratory revealed that she was homozygous for the substitution p.R352H. This mutation was found in all three affected family members and was absent in unaffected individuals. Similar differences are seen between the ten KCN4 families and the other families, suggesting that these differences are due to the specific underlying mutation. Seven families have p.R352H, whereas the other families had novel mutations of KCN4. The only known pathogenic variant in KCN4 that results in hemolytic anemia is the substitution p.R352H. This mutation was found in all three affected family members and was absent in unaffected individuals. This mutation is associated with increased erythrocyte apoptosis and decreased ATP production. The proband's daughter has had mild persistent hemolytic anemia since birth. Her peripheral blood smear showed a few stomatocytes, target cells, and few fragments. At age 39, she has mild splenomegaly and no gallstones. Her 4-year-old daughter is not affected and has normal hemoglobin of 11.8 g/dl at 1.5 years of age. The proband's son, age 34, has more severe hemolytic anemia and hyperbilirubinemia, with an average hemoglobin value of 9.3 g/dl and a total bilirubin of 1.1 mg/dl. He has had iron overload, prompting initiation of iron chelation therapy at age 32. He developed iron overload, prompting initiation of iron chelation therapy at age 32. He has no thrombotic complications.

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Previous clinical reports highlight phenotypic variability both within and between the ten KCN4 families. It is possible that some differences are due to the specific underlying mutation. Seven families have p.R352H, whereas the other families had novel mutations of KCN4. The only known pathogenic variant in KCN4 that results in hemolytic anemia is the substitution p.R352H. This mutation was found in all three affected family members and was absent in unaffected individuals. This mutation is associated with increased erythrocyte apoptosis and decreased ATP production. The proband's daughter has had mild persistent hemolytic anemia since birth. Her peripheral blood smear showed a few stomatocytes, target cells, and few fragments. At age 39, she has mild splenomegaly and no gallstones. Her 4-year-old daughter is not affected and has normal hemoglobin of 11.8 g/dl at 1.5 years of age. The proband's son, age 34, has more severe hemolytic anemia and hyperbilirubinemia, with an average hemoglobin value of 9.3 g/dl and a total bilirubin of 1.1 mg/dl. He has had iron overload, prompting initiation of iron chelation therapy at age 32. He developed iron overload, prompting initiation of iron chelation therapy at age 32. He has no thrombotic complications.
large family with the p.V282M mutation had well-compensated anemia with near-normal hemoglobin levels [5]. In contrast, the proband of another family with p.R352H required transfusion in utero and after preterm birth [3]. Other cases, including those with p.R352H, had variable transfusion requirements during childhood, typically without the ongoing need for transfusion support during adulthood. The majority developed cholelithiasis requiring cholecystectomy. Iron overload was relatively common, though variable within and between families. As in our family, many reported cases underwent splenectomy before a formal diagnosis was made, and splenectomy failed to improve the disease features [2,5].

Although the small numbers of reported DHSt cases, especially with KCNN4 mutations, limit conclusions, differences between the phenotypes of PIEZO1 versus KCNN4 gene mutations are emerging [2–6]. This is particularly the case with regard to the frequency of iron overload, regardless of transfusion regimen, and post-splenectomy venous thromboses. In a retrospective review by Picard, et al. of 126 patients, including twelve from six KCNN4 families, the mean ferritin in KCNN4 patients at diagnosis was 1702 ng/ml, compared to 656 ng/ml for patients with PIEZO1 mutations [2]. Notably, all eight splenectomized PIEZO1 patients in that review developed subsequent thrombotic events requiring long-term anticoagulation. In contrast, none of the four reported splenectomized KCNN4 patients experienced thrombotic events, despite a mean of 26.5 years post-splenectomy. Other case reports with long-term follow-up post-splenectomy, including the one presented here, further support this difference between the hypercoagulable state post-splenectomy for PIEZO1 patients as compared to the absence of thrombotic risk in KCNN4 mutated DHSt [2,5]. Increased understanding of the phenotypic differences, only possible through appropriate diagnosis and reporting of DHSt that results from both KCNN4 and PIEZO1 mutations, is essential to ensure appropriate treatment of these patients.

The recognition and diagnosis of DHSt remain challenging. Not only are the phenotypic patterns different, both between and within PIEZO1 and KCNN4 mutations, but the defining laboratory values also differ. The characteristic findings of red cell dehydration, with an elevated mean corpuscular hemoglobin concentration (MCHC), decreased osmotic fragility, and a left-shifted osmotic gradient ektacytometry, are often absent in patients with KCNN4 mutations [2]. In the family reported here, as well as others, stomatocytes were infrequent, no xeroocytes were identified to assist in diagnosis, and the MCHC was normal. Furthermore, the typical features described for the disease process of DHSt are largely influenced by the disparity in case numbers, which are heavily weighted toward PIEZO1 mutations.

As DHSt due to a Gardos channel KCNN4 mutation is likely to be much more prevalent than reported, confirmation is critical to counsel patients and families appropriately, avoid unnecessary splenectomy and recognize iron overload early in the course of this disorder. Increased recognition of this disease process could also lead to significant treatment advances, such as the use of the selective Gardos channel inhibitor, Senicapoc. This medication has been shown to improve hemolysis in patients with sickle cell disease and might be used to prevent red blood cell dehydration due to a gain of function mutation in KCNN4 [1]. The recognition of this specific disease entity will require a high level of clinical suspicion, plus the inclusion of the KCNN4 gene mutation in commercially available hemolytic anemia panels to appropriately investigate those patients with chronic non-spherocytic hemolytic anemia.

CONFLICT OF INTEREST
The authors have declared no conflict of interest.

ETHICS APPROVAL
This study was performed with IRB approval (Study 00009292), informed consent, and ethics approval as specified by the Human Subjects Protection Office of the Milton S Hershey Medical Center of the Pennsylvania State University.

DATA AVAILABILITY STATEMENT
Supplemental exome sequence data are available on reasonable request to Dr. Carrel.

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