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CASE IMAGE

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Hereditary spherocytosis without pronounced spherocytes on the peripheral blood smear

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A boy with a normal birth weight (3480 g) was admitted to the neonatal ward because of the increased risk on hypoglycemia due to maternal diabetes mellitus. On the second day, jaundice was noted and the total plasma bilirubin raised to 14.6 mg/dl, for which he received phototherapy for 4 days. Since his father had been diagnosed with hereditary spherocytosis (HS) and other causes were ruled out, hyperbilirubinemia was presumably caused by HS.

Around 3 months, the boy returned to the pediatrician for diagnostic work-up for HS. Results from the routine laboratory are shown in Table 1. EMA staining was slightly decreased (78%; reference >85%) and osmotic gradient ektacytometry showed mildly decreased EImax, Ohyper, and Omin. A Next Generation Sequencing (NGS) panel, including SLC4A1, SPTA1, SPTB, ANK1, and EPB4.2 revealed a heterozygous c.3386G>T (p.S1129I) missense variant in the Ankyrin-1 (ANK1) gene, which was considered a variant of unknown significance.

To investigate the pathogenicity of this ANK1 variant, blood was drawn from father, mother, and grandparents from father's side. Both father and grandmother from the index patient claimed to be diagnosed with spherocytosis more than 20 years ago, which data were not available anymore. Grandmother underwent splenectomy, which relieved her severe symptomatic hemolytic anemia. In contrast, father only experienced mild symptoms during childhood, but was free from any complaints for over 15 years. The investigation included a complete blood count, levels of bilirubin and haptoglobin, EMA staining, osmotic lysis test, and osmotic gradient ektacytometry. Furthermore, the ANK1 gene was screened for the c.3386G>T variant.

The peripheral blood smear from the index patient, father, and grandmother showed a few dense and spherical erythrocytes (Figure 1A,B,C), but those may be missed during a routine examination of a peripheral blood smear. Father had slight anemia with reticulocytosis, slightly elevated plasma bilirubin and decreased haptoglobin (Table 1). Normal routine laboratory investigations in grandmother were most likely due to effective splenectomy. In both father and grandmother, the osmotic gradient ektacytometry showed mildly decreased values for EImax, Ohyper, but Omin was normal, unlike typical HS (Figure 1D).[1] Other relatives showed normal osmotic deformability. EMA staining was normal in all family members. Sequencing confirmed that only father and grandmother were carrier for the ANK1 c.3386G>T variant.

Taken together, the newly identified ANK1 c.3386G>T variant is present in all affected family members with signs of spherocytosis. Both index patient and his father do not have clinical signs of hemolytic anemia and do not require any therapy. However, grandmother was treated with multiple erythrocyte transfusions in the past until she underwent a splenectomy. These differences in clinical severity among family members confirms the heterogeneity of HS,[2,3] however we cannot exclude an underlying defect that results in a more severe clinical presentation. Diagnosing HS solely on a peripheral blood smear showed to be difficult, but genetic testing in combination with osmotic gradient ektacytometry show their added value in contributing to this diagnosis. These tests are not widely available on routine diagnostic laboratories, but the analysis may be considered in case of inconclusive laboratory findings.

TABLE 1 Routine laboratory investigations

Test	Index patient	Father	Mother	Grandmother
Hemoglobin (g/dl)	10.0 Ref: 9.7–12.4	13.2 Ref: 13.7–17.7	13.4 Ref: 12.1–16.9	13.9 Ref: 12.1–16.9
RBC ($\times 10^{12}$/L)	3.46 Ref: 3.42–4.8	3.99 Ref: 4.5–5.8	4.49 Ref: 4.0–5.5	4.42 Ref: 4.0–5.5
MCV (fl)	81 Ref: 80–100	96 Ref: 80–100	92 Ref: 80–100	91 Ref: 80–100
MCH (pg/cell)	28.9 Ref: 24.2–28.9	33.1 Ref: 27.4–33.8	29.8 Ref: 27.4–33.8	31.4 Ref: 27.4–33.8
MCHC (g/dl)	35.6 Ref: 30.6–35.5	34.6 Ref: 30.6–35.5	32.2 Ref: 30.6–35.5	34.5 Ref: 30.6–35.5
RDW (%)	16 Ref: 12.3–14.3	15.5 Ref: 12.3–14.3	12.9 Ref: 12.4–15.1	12.4 Ref: 12.4–15.1
Reticulocytes (%)	2.3 Ref: 0.86–1.36	4.8 Ref: 0.86–1.36	1.23 Ref: 0.86–1.36	1.82 Ref: 0.86–1.36
Immature reticulocyte fraction (%)	10.4	18.3	9.2	8.1
Spherocytes (%)	0.8	1.4	0.2	0.3
Bilirubin, total (mg/dl)	n.a.	1.1 Ref: <1.0	0.2 Ref: <1.0	0.3 Ref: <1.0
Bilirubin, direct (mg/dl)	n.a.	0.43 Ref: <0.29	0.08 Ref: <0.29	0.18 Ref: <0.29
Lactate dehydrogenase (U/L)	n.a.	262 Ref: <250	242 Ref: <250	216 Ref: <250
Haptoglobin (g/L)	n.a.	<0.1 Ref: 0.3–1.8	1.1 Ref: 0.3–1.8	0.9 Ref: 0.3–1.8

Note: The average number of spherocytes was determined in >1000 erythrocytes per slide by three independent observers.
Abbreviations: Hb, hemoglobin; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean corpuscular volume; n.a., not available; RBC, red blood cells; RDW, red blood cell distribution width; Ref, reference interval.

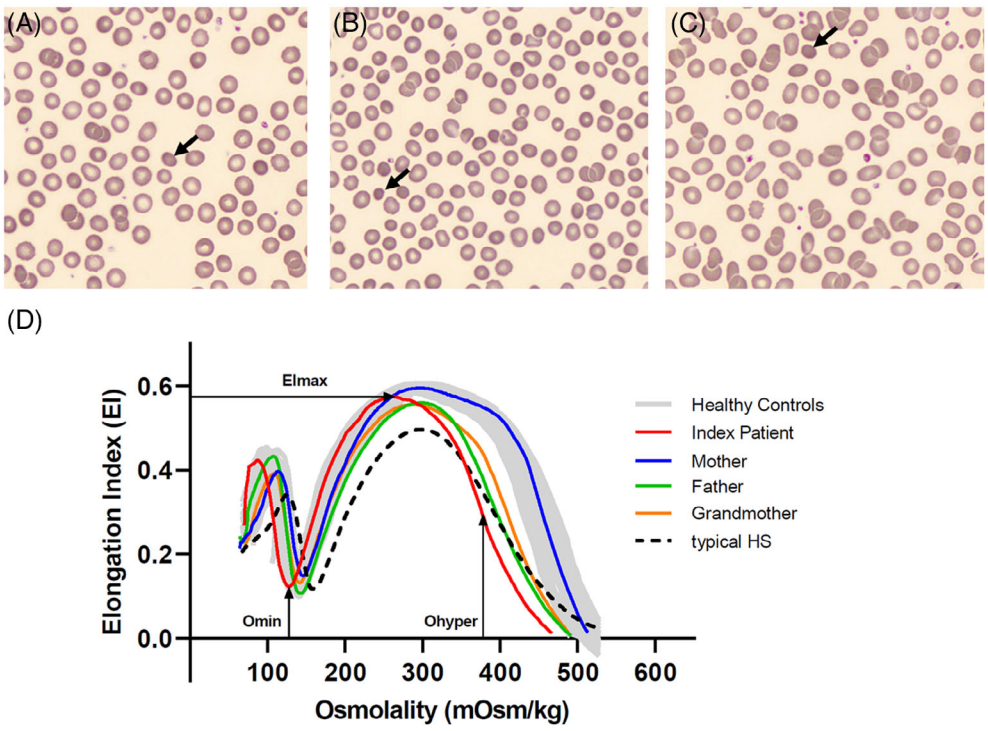

FIGURE 1 A peripheral blood smear from index patient (A), father (B), and grandmother (C). The black arrows indicate dense and spherical erythrocytes. The slides were prepared with a Sysmex SP-50 slide maker and stainer according to the manufacturer's protocol. The slides were stained with a May-Grünwald Giemsa staining. (D) Elongation Index (EI) of erythrocytes measured in an osmolality gradient. The gray area is based on the osmoscans from 34 healthy controls. Index patient (red), mother (blue), father (green), grandmother (orange), and a typical patient with HS (black dotted) are plotted in the graph

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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