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Hereditary xerocytosis: Diagnostic considerations

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To the Editor

Hereditary hemolytic anemias due to primary abnormalities of the red blood cell (RBC) membrane were initially described in relation to their distinctive morphology on peripheral blood smear. The application of Next-Generation sequencing methodology has led to identification of a large number of disease-causing genetic variants associated with specific disorders. There is often considerable clinical and laboratory heterogeneity among individuals with the same genetic variant, sometimes even among family members, suggesting the presence of modifying factors.

We describe a family with clinical and laboratory characteristics of hereditary xerocytosis (HX) associated with an apparently conservative novel variant, L2023V, in PIEZO1, which codes for a mechanosensitive nonselective cation channel. Functional analyses demonstrated a gain-of-function phenotype for the mutant PIEZO1 channel, confirming the pathogenicity of the variant and its causative role for HX. We discuss the utility of specialized testing in
evaluation of patients with hemolytic anemias including ektacytometry, intracellular cation determination, genetic testing, and, when necessary, functional analyses. Obtaining phenotypic and genotypic data from available family members is also a key to assist with the diagnosis.

The proband was a 15-year-old Caucasian male referred for a second opinion on the diagnosis of possible hereditary spherocytosis (HS) with a lifelong history of well-compensated chronic hemolysis. He had history of neonatal hyperbilirubinemia and had continued to experience intermittent jaundice and occasional fatigue but never received a RBC transfusion. He had been offered splenectomy prior to seeking consultation. Peripheral blood smear showed occasional stomatocytes and few spherocytes (Figure 1A). The proband’s three siblings (ages 13, 17, and 19 years) and mother also carried the diagnosis of well compensated chronic hemolysis, thought to be due to HS. Hemoglobin values and RBC indices, remarkable for elevated mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) in most affected family members, are shown in Figure 1B. Of note, all affected family members had well compensated hemolysis with high reticulocyte counts and normal to high hemoglobin values. All three siblings also had history of neonatal jaundice treated with phototherapy and continued to experience some degree of jaundice. The mother and two older brothers had a history of cholecystectomy. The sister had a history of hydrops fetalis including pericardial effusion and ascites that spontaneously resolved.

To begin to clarify the diagnosis, ektacytometry was performed on blood samples from the proband and his parents (Figure 1C). Ektacytometry is used to evaluate RBC deformability under sheer stress at a continuous osmotic gradient and yields distinct patterns for HS and HX.\(^1\) The proband’s father had an essentially normal ektacytometry curve while the patient and his mother had decreased \(O_{\text{min}}\) and decreased \(O_{\text{hyp}}\) reflecting RBC dehydration compatible with HX.

To further define RBC hydration status, we used flame emission spectroscopy to determine intracellular cation values for the proband and his parents (Figure 1D). The proband’s father demonstrated normal intracellular cations. The proband and his mother had low \(K^+\) indicative of the RBC dehydration characteristic of HX. All three of the siblings had an ektacytometry profile similar to the proband’s profile and low intracellular \(K^+\) values similar to those of the proband (data not shown).

Using a Next-Generation sequencing panel containing 32 hemolytic anemia-related genes (Supporting Information Table S1), the patient was found to be heterozygous for a novel conservative PIEZO1 variant c.6067C>G (p.L2023V), predicted by the SIFT, PolyPhen-2, and MutationTaster algorithms to be “possibly damaging.” All family members were enrolled in our IRB-approved Hereditary Hemolytic Anemia study and consented to provide blood samples for sequencing. All three siblings of the proband and their mother were also heterozygous for the PIEZO1 L2023V allele (Figure 1B).

Human PIEZO1 is a 2521 amino acid (287 kDa) protein estimated to have 14–18 transmembrane domains. The L2023V mutation is located in the carboxy-terminus as are the...
majority of pathogenic HX-associated PIEZO1 mutations, in a peripheral helix of the putative ion conducting pore.\textsuperscript{2} There is a very high degree of conservation across species around the 2023 site\textsuperscript{3} (Figure 1E). The clinical history, laboratory findings, and cosegregation of the variant in affected family members suggest that even a conservative substitution of valine for leucine (amino acids with hydrophobic side chains differing by a single carbon in chain length) might cause abnormal PIEZO1 function. To analyze the possible effects of this variant, we performed functional studies as previously described and detailed in Supporting Information.\textsuperscript{4} Stably transfected HEK293 cells expressing either PIEZO1 wild-type (WT) or PIEZO1 L2023V mutant were used for whole-cell patch clamp studies in which mechanical force was applied to the cell surface using a glass probe while monitoring transmembrane currents at −80 mV. Recordings showed channel kinetic differences between the PIEZO1 mutant and WT. Fitting of mechanically activated inactivation currents with mono-exponential function demonstrated a slower inactivation for PIEZO1 L2023V relative to PIEZO1 WT, with a 1.6-fold increase in inactivation time constant (tau) in the mutant PIEZO1 (Figure 1F), similar to other HX-associated gain-of-function PIEZO1 variants.\textsuperscript{5}

It is important that patients with chronic hemolysis, with or without anemia, who present with elevated MCHC be carefully evaluated before assigning the diagnosis of HS. The presence of peripheral blood stomatocytes and/or target cells indicates the need for evaluation for HX. If necessary, this testing may have to be completed at specialized centers with expanded diagnostic laboratory capabilities. Correct diagnosis will direct monitoring and, if necessary, treatment for iron overload\textsuperscript{6} and protect from splenectomy, which is contraindicated in HX due to increased risk of thromboembolic complications.\textsuperscript{7} When the phenotype is ambiguous, genetic testing using appropriately designed panels can provide comprehensive and cost-effective diagnosis, even in patients requiring transfusions. When novel variants or combinations of variants are detected, careful correlation of family history, clinical information, laboratory data, and genetic information from available family members is required. In complicated cases where results remain ambiguous, functional studies may be necessary to elucidate the correct diagnosis.

Preliminary results from this work were presented at the American Society of Hematology Annual Meeting in San Francisco, CA on December 9, 2014.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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References

FIGURE 1.
Evaluation of RBC phenotype in the proband (A–D) and of functional significance of the PIEZO1 L2023V mutation (E,F). A, Wright-stained peripheral blood smear. Large arrows indicate stomatocytes; small arrow points to a spherocyte. B, Pedigree chart of proband’s family with hemoglobin values and RBC indices for affected family members. P = proband. Reference ranges: Hgb 13–16 g/dL male, 12–16 female; MCV 78–94 fL; MCHC 31–36 g/dL; ARC 39–100 × 10^3/µL. C, Ektacytometry osmoscan showing that the proband’s father had an essentially normal profile while the proband and the proband’s mother had an HX profile with decreased O_{min} and O_{hyp}. D, Intracellular cation values determined by flame
emission spectroscopy indicating decreased K+ in the proband and his mother. E, Sequence surrounding L2023 in several species illustrates strong conservation in this region. 

F, Delayed channel inactivation of the L2023V mutant. Top graph: Whole cell patch clamp at ~80 mV. Representative traces of mechanically activated currents recorded from HEK293 cells expressing wild type PIEZO1 (PIEZO1-WT; black trace) or mutant PIEZO1 (PIEZO1-L2023V; red trace). Traces were normalized to the peak current. Maximum current responses for each construct were overlaid for inactivation kinetics purposes. Bottom graph: Average of inactivation time constant (tau, ms) from mono-exponential fits for PIEZO1-WT (black) and mutant PIEZO1-L2023V (red). The means were compared using two-tailed Student’s t-test (P < .0001). Bars represent mean ± SEM; n = number of cells tested [Color figure can be viewed at wileyonlinelibrary.com]