P1537 HEREDITARY ANEMIA CAUSED BY MULTILOCUS INHERITANCE OF PIEZO1, SLC4A1 AND ABCB6 MUTATIONS: A DIAGNOSTIC AND THERAPEUTIC CHALLENGE.

Topic: 28. Enzymopathies, membranopathies and other anemias

Barbara Eleni Rosato1, 2, Seth L. Alper3, Giovanna Tomaiuolo4, 2, Roberta Russo1, 2, Achille Iolascon1, 2, Immacolata Andolfo1, 2

1 Dip. di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli - Federico II, Napoli, Italy; 2 CEINGE - Biotecnologie Avanzate, Napoli, Italy; 3 Dept. of Medicine, Harvard Medical School, Boston, Massachusetts, USA, Renal Division and Molecular and Vascular Medicine Division, Beth Israel Deaconess Medical Center, Boston, United States; 4 Dip. di Ingegneria Chimica, dei Materiali e della Produzione Industriale, Università degli Studi di Napoli - Federico II, Napoli, Italy

Background:

Red blood cell (RBC) disorders encompass a vast group of hereditary hemolytic anemias (HHA) that differ in clinical, morphologic, and molecular features. Subtypes include RBC disorders caused by altered (i) membrane structural organization and (ii) transport function. The most common form of the first group is Hereditary Spherocytosis (HS) presenting anemia, reticulocytosis and splenomegaly due to mutations in SLC4A1, ANK1, SPTA1, SPTB, and EPB42 genes. The second group is represented by Dehydrated Hereditary Stomatocytosis (DHS) characterized by dehydration of RBC and iron overload, due to mutation in PIEZO1 gene (90% of cases). DHS may present as syndromic form with familial pseudohyperkalemia (FP), a red cell trait characterized by loss of K+ at low temperatures. FP can be isolated and due to mutations in ABCB6 gene. The heterogeneity of these disorders often hampers correct clinical diagnosis. Genetic testing is now a suitable approach for differential diagnosis and identification of polygenic conditions.

Aims:

We here investigate a patient with HHA using genetic and functional tests to demonstrate the complexity of multilocus inheritance and the need for precise genetic diagnostics.

Methods:

Targeted Next Generation Sequencing (t-NGS), Western Blot (WB), ektacytometry, ion flux assay.

Results:

The 24 years old proband, presented moderate anemia, jaundice, and splenomegaly. Blood smear revealed the presence of stomatocytes, target cells, and rare ovalocytes. Pink and acidified glycerol lysis tests were positive, leading to an initial clinical diagnosis of HS. The patient was treated by total splenectomy but, after surgery, she experienced portal vein thrombosis and anemia worsened. Furthermore, she developed severe iron overload and hyperkalemia. Indeed, studies of RBC cation content revealed high intracellular Na+ and low intracellular K+, with increased leak flux at 37°C, suggesting a FP.

The proband was referred to our unit at 38 years old and tested for SLC4A1 gene. We found a heterozygous missense variant p.Thr686Met, predicted to impair splicing, and reported as likely pathogenic. Analysis of RBC membranes by WB, revealed a reduction of Band 3 expression, confirming the pathogenicity of the variant.

The worsening of anemia prompted re-evaluation of the case by t-NGS for RBC defects. A heterozygous nonsense variant in the ABCB6 gene p.Tyr471*, and a heterozygous missense variant p.Val598Met in the PIEZO1 gene were found. Thus, the occurrence of the ABCB6 variant explained the hyperkalemia and the temperature studies, while the
presence of the \textit{PIEZO1} variant explained the altered permeability of the RBC, the hepatosiderosis, and the thrombosis after splenectomy. Finally, the ektacytometry analysis confirmed the multilocus inheritance, revealing a left shifted osmotic curve (typical of DHS) with a slightly decreased DiMax (typical of HS).

\textbf{Summary/Conclusion:}

After 20 years, the patient received a complete diagnosis of multilocus inheritance of HS, DHS and FP due to mutations in \textit{SLC4A1}, \textit{PIEZO1}, and \textit{ABCB6} genes. This paradigmatic clinical case underlines the importance of a correct clinical assessment and genetic diagnosis to guide the personalized clinical management of the patient. An early diagnosis of DHS would have prevented the splenectomy and the following complications. This case confirms that the use of NGS-based testing in the diagnostic workflow of hereditary anemias is crucial to make a complete differential diagnosis.