Hereditary spherocytosis and allied disorders

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Take home messages
- Erythrocyte membrane disorders embrace a group of chronic diseases characterized by high genetic and phenotypic variability.
- Differential diagnosis, classification, and patient stratification among erythrocyte membrane disorders are often challenging.
- The increasing understanding of molecular genetics of erythrocyte membrane disorders highlights the problem of establishing a correct phenotype-genotype correlation.

Introduction

Erythrocyte membrane disorders are a subtype of hereditary hemolytic anemias that embrace a highly heterogeneous group of chronic diseases with high genetic and phenotypic variability. They are caused by mutations in genes encoding for various transmembrane or cytoskeletal proteins of red blood cells (RBCs), resulting in decreased erythrocyte deformability and shortened red cell survival. Extensive studies on RBC membrane have allowed the comprehension of both the structure and the function of this subcellular compartment. However, although the molecular bases of most cases of RBC membrane alteration have been defined, there are still several aspects to be learned about the molecular basis of membrane disorders (Table 1).

Nevertheless, with the rapid development and broad application of next-generation sequencing (NGS) diagnostic technologies, many novel pathogenic variants have been identified in RBC membrane-related genes. To note, most of the identified mutations are sporadic and specific to individual patients, or their families, highlighting, even more, the problem of the remarkable allelic heterogeneity, beyond the genetic one, in determining the phenotypic variability.

Current state of the art

The erythrocyte membrane plays a critical role in the function and structure of the RBCs. It provides the unique biconcave disk shape that enables the erythrocyte to undergo repeated distortion into the microvasculature and the spleen during its 120-day life span.\(^1,2\) The erythrocyte membrane is a barrier with selective permeability to maintain pH homeostasis, the membrane exchanges of chloride and bicarbonate anions, and it actively controls the cation and water content of the erythrocyte. It is a complex structure composed of a fluid lipid bilayer stabilized by an underlying 2-dimensional membrane skeleton, which maintains the integrity of the biconcave disk shape of the erythrocyte. The skeleton is responsible for the deformability by sustaining the shear stress come across the tiny capillaries of the microcirculation and in the spleen. Multiple protein-protein interactions between (i) integral membrane proteins within the lipid bilayer, (ii) peripheral proteins, and (iii) linker proteins, which link the cytoskeleton to the transmembrane proteins, determine the structure of the erythrocyte membrane. Alterations of both vertical and horizontal interactions may have clinical consequences.\(^1,4\) The vertical one involves the cytoplasmic domains of band 3 (chloride/bicarbonate exchanger) and RhAG (ammonium and carbon dioxide transporter), ankyrin (protein that links the integral membrane proteins to the underlying spectrin-actin cytoskeleton), protein 4.2 (ATP-binding protein, which regulates the association of band 3 with ankyrin) and β-spectrin. The horizontal linkages in the skeletal membrane network include spectrin self-association (α-spectrin with β-spectrin) and spectrin-actin junctional complex. Alterations of both vertical and horizontal interactions and due to mutations in the various membrane and skeletal proteins.\(^1,2\)

Hereditary spherocytosis (HS) is the most common nonimmune hereditary chronic hemolytic anemia and is triggered by the impairment of the vertical interactions.\(^1,2\) The clinical manifestations of HS vary widely and consists of chronic hemolysis with anemia, jaundice, reticulocytosis, gallstones, splenomegaly as well as spherocytes on peripheral blood (PB)
The impairment of horizontal interactions leads to hereditary elliptocytosis (HE) characterized by the presence of elliptical-shaped erythrocytes (elliptocytes) on the PB smear associated with variable clinical manifestations. Most patients present no anemia or hemolysis. HE can be due to mutations, inherited in autosomal dominant manner, in EPB41, SPTA1, and SPTB genes that lead to severe damage in the association of spectrin dimers/tetramers. A subtype of HE is hereditary pyropoikilocytosis (HPP), a rare severe hereditary anemia characterized by poikilocytosis and fragmented erythrocytes and microspherocytes. It is due to either homozygous or compound heterozygous mutations in SPTA1 leading to severe disruption of spectrin self-association.\(^{5,7,8}\)

Regarding the treatment, the first line is often only supportive care as phototherapy in neonatal patients and transfusions in severe cases. Similarly, the severe cases required transfusions in adulthood, mostly during aplastic or hemolytic crises. Splenectomy is beneficial in several cases of HS and HE/HPP, resulting in an increased life span of RBCs.\(^{1,9}\)

Although the workflow to diagnose RBC membrane disorders is a standard clinical practice, differential diagnosis, classification, and patient stratification among these diseases are often very difficult. Indeed, the variety of unspecific and overlapping phenotypes often hampers correct clinical management of the patients. For example, it is classically reported the problem for differential diagnosis between HS and congenital dyserythropoietic anemia type II.\(^{1,10}\) In this context, the genetic testing represents a precious tool for both the diagnosis and the management of these patients.

**Future perspectives**

With the widespread use of NGS diagnostic technologies, several novel mutations have been identified. Indeed, approximately 30% of new pathogenic variants have been annotated in HS-HE-related genes in the Human Gene Mutation Database in the last 4 years (2015–2018). NGS data have shown that in some cases mutations in more than 1 causative gene were identified. Indeed, multigene diagnostic approach allowed the identification of polygenic conditions, in which the coinheritance of multiple disease genotypes could explain the phenotypic variability observed in these patients.\(^8\) To note, the exome approach demonstrated that at least 3% of the hereditary diseases are oligogenic conditions. Moreover, NGS approaches could also help in defining the inheritance of genetic modifiers. Thus, the increasing knowledge about molecular genetics of HS and allied disorders highlighted the problem of an augmented difficulty in the establishing a correct phenotype-genotype correlation.\(^{3}\) In our experience, the multigene diagnostic approach allowed confirming the clinical suspicion in 84.6% of patients initially suspected of hereditary stomatocytosis or HS.\(^8\) This observation suggests that the clinical and biochemical definition of these patients represents the first-line step to diagnose. However, genetic testing is essential since the phenotypic diagnosis may not be readily available, for example, in patients requiring frequent transfusions, and it often does not predict disease course or severity.

**References**

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**Table 1**

| Disease | Subtype | Phenotype MIM Number | Inheritance | Gene | Location |
|---------|---------|----------------------|-------------|------|----------|
| HS      | 1       | 182900               | AD          | ANK1 | 8q11.21  |
|         | 2       | 616649               | AD          | ANK1 | 8q11.21  |
|         | 3       | 270970               | AD          | SPTB | 1q23.3   |
|         | 4       | 612653               | AD          | SPTA1| 1q23.1   |
|         | 5       | 612690               | AD          | SLC4A1| 1q23.3   |
| HE      | 1       | 611804               | AD          | SPTA1| 1q23.1   |
|         | 2       | 136600               | AD          | SPTB | 1q23.1   |
|         | 3       | —                   | AD          | SPTB | 1q23.1   |
| HPP     | —       | 266140               | AR          | SPTA1| 1q23.1   |

**AD** = autosomal dominant, **AR** = autosomal recessive, **HE** = hereditary elliptocytosis, **HPP** = hereditary pyropoikilocytosis, **HS** = hereditary spherocytosis.
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