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Novel Variants in Hemoglobinopathies: Genomic Approaches and Clinical Relevance

*** V.Vijayalakshmi, Shyam Sudarsan.S[1], Dr.Vaishnavi.A[2], Dr.K.P.Sivakumar[3]**

[1]Shyam Sudarsan.S, Assistant Professor, School of Pharmacy, SBV Chennai, Sri Balaji Vidyapeeth (Deemed to be university) Pondicherry 607402. INDIA

[2]Dr. A. Vaishnavi, Assistant Professor, Department of Pharmacology, Faculty of Pharmacy, Sree Balaji Medical College and Hospital Campus, Bharath Institute of Higher Education and Research, Chromepet, Chennai-44

[3]Dr.K.P.Sivakumar, Librarian, Kamaraj College of Engineering and Technology, (An Autonomous Institution-Affiliated to Anna University, Chennai), S.P.G.C.Nagar, K.Vellakulam

[*]**Corresponding Author**

[*]V.Vijayalakshmi, Assistant Professor, Department of Pharmacology, Faculty of Pharmacy, Sree Balaji Medical College and Hospital Campus, Bharath Institute of Higher Education and Research, Chromepet, Chennai-44

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ABSTRACT:

One of the most important and critical red blood cell disorders is dysfunction and deformation of membrane structure, which affects the metabolic and biological red blood cell functions. On the other hand, the basic causes of these problems are the genetic mutations in the production of proteins that correlated to the structure and receptors of cells. The diagnosis methods and techniques are the other essential points that focus most scientists on. In this systematic review, the article pointed to the key title, which is the diagnosis of novel genes with different techniques and methods. The result of articles studies that were published in the last decades underlined the types of techniques such as Whole-Exome Sequencing, Quantitative Real-Time PCR, Targeted Next-Generation Sequencing, and Sulphate-Polyacrylamide Gel, which investigated the different membraned gene mutations that are novel and correlated to the genes that make the structures and functions of red blood cells such as hereditary spherocytosis, hereditary elliptocytosis, hereditary pyro poikilocytosis. In addition, the hereditary membrane disorders correlated to others such as hereditary spherocytosis has relationship with vitamin B12, immunodeficiency.

Introduction

Normal erythrocyte's lipid bilayer membrane contains proteins that stick to the cytoskeleton and give it a normal biconcave form. This membrane maintains structural integrity, provides the ideal surface/volume ratio, and has sufficient ion permeability (1, 2). Red blood cell membrane diseases are a diverse collection of hereditary conditions brought on by specific gene mutations that affect the proteins that connect the lipid bilayer with the membrane cytoskeleton. Hemolysis results from a lack or malfunction of these proteins, which reduces the red blood cells (RBC) deformability in the splenic entrapment and narrow capillary beds (1). Some of the inherited Red Blood Cell disorders are due to altered membrane function have been identified such as hereditary spherocytosis (HS) (3) hereditary pyropoikilocytosis (HPP) (4) hereditary elliptocytosis (HE) (5) and hereditary stomatocytosis (HSt) (6). The common inherited red blood cell (RBC) membrane condition known as hereditary spherocytosis (HS) is characterized by a wide range of genetic patterns and clinical symptom severity (7). RBC membrane protein gene analysis by genetic testing is becoming a useful diagnostic adjunct to traditional testing (7, 8). The five major RBC membrane protein genes (which span 40–50 exons) may be analyzed for mutations using time-consuming and expensive conventional molecular approaches. An important benefit of next-generation sequencing (NGS) technology is that it allows for a thorough examination of mutations in each of these genes at the same time (7, 9).

It is now feasible to identify the causal gene in HS because to the advent of high-efficiency genome sequencing techniques like NGS (7). Osmotic gradient ektacytometry is the gold standard for measuring the deformability of red blood cells, but sadly, only a small number of labs

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have access to the device(10). In certain situations, such as when the screening tests are inconclusive, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) is typically used to confirm the

faulty protein (11). Currently, genetic studies are only carried out under certain circumstances, including a normal EMA binding test or a negative family history. According to recent research, a person's genotype can affect their clinical variability, severity, and evolution throughout the course of their lifetime. As a result, new genetic technologies, such as Next Generation Sequencies, may eventually be incorporated into the diagnosis process (10, 12). In this systematic review, the article is collected to sort the novel genes which correlated to different types of RBC membrane disorders and diseases, which are studied with various methods, laboratory instruments, and techniques of analysis.

Method and material

In this systematic review article, information is collected from famous international hematological and medical journals such as ScienceDirect, PubMed, International of Laboratory Hematology, and American Journal of Hematology. The flowchart (**Figure 1**) describes more details about the steps of filtering which do not depend on electronic methods.

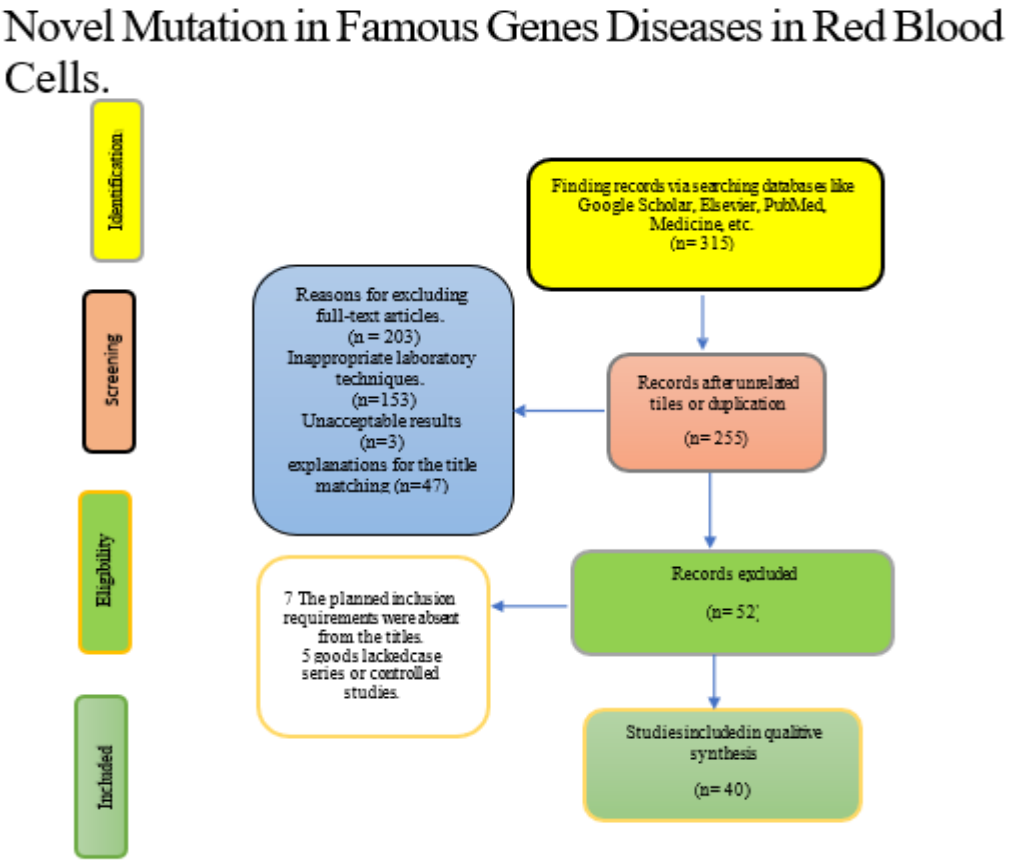

Figure 1. Flowchart of data extraction and search strategy finding

Hereditary Spherocytosis

HS patients have abnormalities in the genes coding of ankyrin (ANK 1), α-spectrin (SPTA1), β- spectrin (SPTB), protein 4.2 (EPB42), band 3 (SLC4A1). Serious recessive HS is caused by biallelic SPTA1 mutations (7, 13). Abnormalities in the membrane components of HS patients are first caused by gene mutations, mostly following an autosomal dominant pattern (7). The HS heterogeneity arises from mutations in ANK1, SPTA1, SPTB, EPB42, and SLC4A1 genes, which can be verified in frequency and mutation type between nations (7). According to mass spectrin is abounded between of them

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(14). The membrane protein abnormality correlated with clinical manifestations of severe HS. As a survey of 300 European patients, about 54% of them were HS with defects of band 3 protein, and in the South Korea, 52% are due to defects of ankyrin protein. Moreover, in the development of severe anemia reported in α-spectrin defects, whereas in mild to moderate anemia patients reported the ankyrin, band 3, and protein 4.2 defects. The types of membrane protein defects in HS vary from regional to inter-population, which makes it hard to diagnose HS (15).

Hereditary Elliptocytosis

One of the haemolytic anemia reasons is HE (16). Which mutations in the spectrum, protein 4.1, or glycophorin C cause RBCs to be oval or elliptical in HE (ovalocytosis), a rare autosomal dominant condition. Except in certain people who are homozygous (hereditary pyre poikilocytosis), hemolysis is often absent or minimal, and anemia is little or nonexistent (17). HE is common worldwide with a higher prevalence in malaria endemic areas, including Southeast Asia (18) West Africa has prevalence reaches 0.6% to 1.6%(16, 19, 20).The main cause of defect in HE is due to dysfunction or deficiency of RBC cytoskeletal proteins, namely, α-spectrin (SPTA1), β-spectrin (SPTB), and protein 4.1R (EPB41R) (**Figure 2**) Homozygous or compound heterozygous allele mutations in the genes underlying the spectrin–protein 4.1R-actin complex weaken the "horizontal" cytoskeletal associations in HPP, resulting in an increase in RBC fragmentation and thermal instability, poikilocytosis with micro-spherocytosis, and striking microcytosis, with a mean corpuscular volume (MVC) usually less than 60 fL (21).

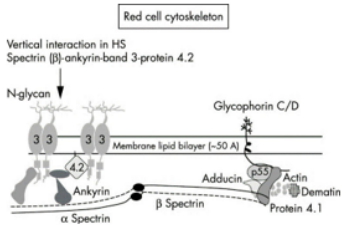

Figure 2. Diagrammatic representation of the cytoskeleton's structural arrangement in RBCs. b the crucial element is spectrin, which includes binding sites for ankyrin and protein 4.1 and forms a heterodimer with another spectrin. Spectrin (a and/or b) is linked to the prevalent protein defects. protein 4.1, band 3 protein, and ankyrin (1).

Hereditary pyro poikilocytosis.

HPP is a heterogeneous inherited affecting RBC membrane and cytoskeletal proteins, resulting in hemolytic anemia. HPP is distinguished by significant poikilocytosis, micro spherocytes, red blood cell fragmentation, and elliptocytes seen on peripheral blood smear. A quantitative deficit in α- spectrin caused by mutations in SPTA1 can result in HPP, significant fetal anemia, and nonimmune hydrops fetalis, which can be treated by intrauterine transfusion (22). Patients with HPP have biallelic mutations in genes encoding cytoskeletal proteins a-spectrin (SPTA1), b- spectrin (SPTB), or protein 4.1R (EPB41). And also, the cause of hemolytic anemia is heterogeneous (severe (18)) congenital disease of the cytoskeletal proteins and RBC membrane. On a peripheral blood smear, HPP is distinguished by elliptocytes (22), poikilocytes, and microspherocytes (20, 23). RBC fragmentation and noticeable poikilocytosis (18, 22).

Hereditary Stomatocytosis

The presence of stomatocytosis is correlated with an increase in circulating reticulocytes (reticulocytosis) and a decrease in intravascular hemolysis and cellular fragility. Clinical signs that are typical include pallor, jaundice, exhaustion, gallstone formation, and splenomegaly(42). Stomatocytosis has two major forms: dehydrated and overhydrated, and dehydrated is the most common, occurring in roughly one out of every 50,000 human births (25). And also, some of the genes linked to stomatocytosis are involved in the transport of Ca2+, K+, Na+ or ion exchanging, and Cl-. For example, hereditary dehydrated stomatocytosis is commonly caused by autosomal dominant genetic changes in ion channel genes KCNN4 or PIEZO1. Overhydrated hereditary stomatocytosis is linked to genetic changes in anion transport genes SLC4A1; SLC2A1(GLUT1), and RHAG. Stomatocytosis rarely can be caused by genetic changes in lipid metabolism genes, such as ABCG5 and ABCG8, which encode sterol transporters(26). other classification of HSt is depends to clinically and genetically relationships to simplify discerption which are extra- hematologic symptoms and non-syndromic forms, characterized by selective involvement of the erythroid system summary HSt with correlated diseases demonstrated in molecular diagnosis indicated in (**Table 2**) (24).

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Table 2. Summary of molecular defected of HSt with association diagnosis and diseases(2).

Genes	Function of its coded proteins	Coding options	Diseases
PIEZO1	Conductors monovalent and divalent cations (Na^{+1}, K^{+1}, Li^{+1}, Cs^{+1}), (Ba^{+2}, Ca^{+2}, Mg^{+2}, and Mn^{+2}.	Mechanoreceptor	in the vascular and renal systems. And also indicated congenital lymphatic dysplasia with the description of loss of function mutations.
ABCB6	blood group antigen system. Leads to an increased cation flux compared to WT protein in an in vitro model of FP in HEK-293 cells.	ATP-binding cassette (ABC) transporters. member of the B (MDR/TAP) subfamily of ABC transporters best known as drug-resistance genes	Increased ABCB6 expression correlates with increased drug resistance in multiple cell lines.
KCNN4	Ca^{+2} dependent K^{+1} channel of intermediate conductance that mediates the major K^{+1} conductance of RBC.	Gardos channel,	Considered as a potential target for therapeutic approaches in different diseases like asthma, sickle cell anemia, atherosclerosis, kidney fibrosis, and autoimmunity.
RHAG	Antigens D and CE Rolld group carrier, Forms the core of the Rh complex—including glycophorin B, CD47, intercellular adhesion molecule-4 (3), and band 3. ammonium or CO2 (carbon dioxide) channel	Rhesus associated glycoprotein (RhAG) membrane glycoprotein	Mutations, Ile61Arg and Phe65Ser, have been described until now in overhydrated hereditary stomatocytosis patients. Moreover, modeling studies described that both mutations dilate a cytoplasmic constriction in the RhAG pore. Wild-type RhAG protein induces a cation flux when expressed in X. laevis oocytes, and the mutants induce a much larger flux, indicating a gain of function.
Band 3 or anion exchanger 1 (AE1, SLC4A1)	Exchanging the anions, Cl^- and HCO^{3-} under physiological conditions, together with carbonic anhydrase and Hb, it is one of the important components of CO2 carrier in RBCs.	Anion Exchanger 1 (AE1) or band 3	Field of function mutations genes are mainly correlated with HS, leading to the loss of linkage between the cytoskeleton and plasma membrane and thus to structural defects. Specifically, patients with milder forms of overhydrated hereditary stomatocytosis have been related to the SLC4A1 mutation Arg730Cys. Mostly, the mutations are located near the transport site of the protein. In addition, a case of DHS with dyserythropoietic has been related to a specific band 3 mutation, Gly796Arg, characterized by the conversion of band 3 from an anion exchanger to a cation transporter.
SLC2A1	Binds stomatin, and may be converted to a L-dehydroascorbate transporter in human erythrocytes and other tissues.	Glucose transporter GLUT1	Various mutations are responsible for this defect of RBC cation content: a deletion of 12 nucleotides leading to Gln282_Ser285del in the 7th membrane spanning helix of the transporter, a substitution of Gly286Asp in the same helix, and a deletion of 3 nucleotides leading to the Ile436del.

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Gallbladder with hereditary spherocytosis

The most common complication of HS is gallstones, which affect approximately 40% of patients who are not splenectomized, and are the main reason for carrying out a cholecystectomy in patients with HS (27). For patients with symptomatic gallstones, the guidelines from the British Committee for Standards in Hematology (BCSH), published in 2004 and updated in 2011, recommended that "the gall bladder should be removed" with or without splenectomy (28). In recent decades, it has been widely and essentially accepted that cholecystectomy should be performed simultaneously for patients with asymptomatic gallstones undergoing splenectomy. Despite the 2011 guidelines,

the value of cholecystectomy is still controversial. The most recent guidelines from the European Hematology Association (EHA) published in 2017 did not give any recommendations for these patients, who account for the majority of patients with HS and cholelithiasis. Furthermore, the progression of gallstones in HS patients who have had their spleens removed is still a mystery. There is a lack of research on the management of HS patients with asymptomatic gallstones. Despite the 2011 recommendations, the value of cholecystectomy is still controversial. The European Hematology Association (EHA) released the most recent guidelines in 2017 that did not recommend these individuals, who make up the majority of patients with cholelithiasis and HS. The development of gallstones in splenectomized HS patients is still unknown. It is uncommon to find research on the treatment of HS patients who have asymptomatic gallstones (27, 28).

Hereditary Spherocytosis with vitamin B12

The laboratory study has shown that HS type 1 correlates with vitamin B12, and the details are summarized in **Table 3** (29). The results of the osmotic fragility test showed a normal figure with hemolysis starting at 0.4% and ending at 0.1%, and the median corpuscular fragility was 4.1% (which is within the normal range of 4–4.5%). Using EMA, a flow cytometric analysis revealed a mean fluorescence intensity (MFI) of 8609 compared to a control of 8429. The patient's MFI (8609) is slightly higher than the control (8429), with only a 2.13% increase in MFI percentage difference (MFI Patient - MFI Control/MFI control) × 100, which is not consistent with HS. However, (MCHC >33 g/dL), the presence of spherocytes, and the family history of the mother undergoing splenectomy at 12 years of age due to a blood disorder (details not available), diagnosis of HS was considered. A genetic analysis performed on ANK1 (NM_000037.4), intron 20, c.2296- 2A>G, was performed and identified as a heterozygous pathogenic AD mutation, which confirmed the diagnosis of HS type 1.(29). Other studies which proved that HS has a correlation with vitamin 12, laboratory points and properties such as growing anemia (Hb 7.7 g/dl) and diverticulitis, bad diet and being experiencing anorexia and diarrhea, fewer amounts of vitamin B12 and folate were found in serum examinations, in spit the history of HS, blood film had a very normal red cell osmotic fragility curve and very few spherocytes. Rather, the blood film displayed oval macrocytes and normocytes, the majority of which had typical center pallor (30).

Table 3: output of summary of laboratory report (4)

Assay	Unite	Quantities
Hb	g/dL	2.9
Neutrophils	%	58.9
Lymphocytes	%	32.5
Eosinophils	%	2.5
Monocytes	%	6.1
Platelet	Lakh	1.08
reticulocyte count	%	0.21
peripheral blood smear	Negative	Atypical
Spherocytes	Present	Few
Direct Coombs test	-	Negative
Total bilirubin	mg/dL	2.42
Direct bilirubin	mg/dL	0.44
Vitamin B12 (160-950pg/mL)	Pg/mL	<159
Mean corpuscular hemoglobin concentration	>33g/dl	34.5

Hereditary Spherocytosis with Immunodeficiency

Adults infected with immunodeficiency virus have a lower incidence of HS. Early detection and treatment are necessary for these patients at the familial level through the use of a test algorithm

(31). In spit the lack of a direct link between HIV and HS, HIV complicating HS has significant effects on clinical presentation and available treatments (32). On the other hand, a significant portion of HIV-positive individuals have anemia while beginning ART. Male sex, bedridden or ambulatory functional status, a CD4+ T cell count <200 cells/mm3, a lower BMI, and a lack of formal education were found to be independent predictors of anemia (33). Clinical features, a positive family history,

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and spherocytes in a peripheral blood smear (15) are all required for the HS diagnosis. Additional confirmatory tests include the osmotic fragility test, auto hemolysis test, flow cytometric-based eosin-5-maleimide (EMA) binding test, and protein analysis employing gel

electrophoresis or mass spectrometry (15) Furthermore, genetic testing analysis of RBC membrane protein genes is emerging as a valuable diagnostic tool in addition to conventional assays (15). The ability to thoroughly examine alterations in each of these genes simultaneously (9, 15) is a significant advantage of next-generation sequencing (34) technology. In order to ascertain the extent of HS among HIV-positive individuals visiting UOGCSH in North West Ethiopia, a cross- sectional study was carried out. The findings of the inquiry showed that HS was present among HIV-positive people (31).

Homolysis in newborn elliptocytosis

Homolysis is a very rare with cause of HS with international distribution. Approximately 0.03% to 0.05% has prevalence in Europe and the United States (19, 20). Newborns by association of laboratory routine assays such as bilirubin, direct and indirect AHG tests, blood group antibodies screen, hemoglobin electrophoresis, G6PD, H inclusion bodies, and blood and urine genetic metabolism screens were negative. Phototherapy and transfusion may be required for infants with severe hemolytic anemia and jaundice. The appearance of elliptical-shaped RBCs in the peripheral blood smear, which can Make up anywhere from 15% to 100% of the total RBCs, is the hallmark feature of common hereditary elliptocytosis. Additionally, spherocytes, stomatocytes, and Poikilocytes (fragmented cells) may be seen on the peripheral blood smear (35). In contrast, an acquired elliptocytosis that is a chronic myeloid neoplasm was abnormal in the long arm of chromosome 20, del(q20), due to cytogenetic alterations, Moreover, this phenotypic feature is seen in myelodysplastic syndrome and other chronic myeloproliferative diseases (36).

Hereditary Pyropoikilocytosis Presenting as Hydrops Fetalis

Mutations in SPTA1 can cause HPP due to a quantitative defect in α-spectrin and can lead to profound fetal anemia (18) and nonimmune hydrops fetalis, which can be managed with intrauterine transfusion (22). Correlates homozygosity of the SPTA1c.6154delG gene variant with RBC dysmorphology and establishes the diagnosis of HPP (22). In a case involving a 26-year-old Amish-Mennonite woman of G4P2102 whose pregnancy was complicated by fetal homozygosity for an SPTA1 gene variant (SPTA1c.6154delG), severe fetal anemia, and hydrops fetalis, four intrauterine transfusions were administered between 26- and 30-weeks' gestation (37).

Diagnostic Protocol for Hereditary Spherocytosis

According to the main British recommendations for the diagnosis and management of HS, confirmatory diagnostic laboratory testing is no longer required for newly diagnosed individuals with a family history of HS and a clinical history of the disease. The primary British guidelines for the diagnosis and treatment of HS (38), state that newly diagnosed patients with a clinical history of HS and family members do not require further testing beyond confirming diagnostic laboratory tests. However, if the diagnosis is unclear, screening methods such as cryohemolysis and the EMA binding test are recommended (38). If the screening findings are unclear or ambiguous, then specialist testing is required, particularly gel electrophoresis investigations of

erythrocyte membranes like SDS-PAGE. The latter is recommended in the following circumstances:

1. When one family history observation is more severe than the RBC morphology.
2. When the RBC morphology is less severe than the clinical presentation.
3. If, before the splenectomy, the diagnosis was not obvious.

Use a simple diagnosis process for HS that was updated in 2021 and incorporates more tests such as mean sphered corpuscular volume and mean reticulocyte volume. It also takes into account the presence of chronic hemolysis (increased indirect bilirubin, reticulocyte count, and LDH, decreased haptoglobin with or without anemia), genetic testing, family history, symptoms (physical examination), jaundice, splenic enlargement, and other laboratory surrogate tests (10, 39).

Tests for Screening

• The Hb level is normal in trait form, 60–80 g/L in severe, 80–120 g/L in moderate, and 110– 150 g/L in

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mild.

- The reticulocyte count is less than 3% in trait form, >10% in severe cases, >6% in moderate instances, and 3% to 6% in mild cases.
- MCHC > 355 g/L is utilized as the limit for HS diagnosis.
- There are a lot of spherocytes.
- Induction of total serum bilirubin occurs, particularly for unconjugated bilirubin.
- MSCV < MCV; mean reticulocyte volume is decreased (cutoff: ≤95.77 fL).

In contrast to the previous guidelines, the most recent one recommends the use of analytical techniques, including the G6PD level measurement, acidified glycerol lysis test, Coomb's test, -5′- maleimide binding test EMA (10), Osmotic Fragility Test, and genetic testing. ICSH, on the other hand, sought an algorithm for HS suspicion. RDW-CV and parameters obtained from the reticulocyte channel are recommended in order to confirm the hemolysis. A blood smear is done to examine for the presence of spherocytes or other morphological abnormalities. Cryo-hemolysis and EMA testing are carried out if the HS diagnosis is still questionable. There are two options in this situation. Both tests are negative, therefore no more testing is necessary. Results of the ektacytometry test are either positive or questionable. Additionally, the diagnosis can be maintained if the patient's anamnesis is characteristic for HS. SDS-PAGE is used to rule out CDA II, validate the HS diagnosis, and identify the protein mutation in cases of questionable anamnesis. Only when there is a discrepancy between the clinical profile and the analytical data or when there is a chronic transfusion is molecular analysis carried out (39).

Genetic Laboratory Diagnosis

The standard screening test is used in conjunction with gene expression analysis of HS to increase the accuracy of HS diagnosis. In actuality, some labs employ the high-resolution melting curve approach, but occasionally it has a few drawbacks, including inadequate A/T or G/C base alteration detection. As a result, NGS, the new genetic screening and diagnosis technology, largely replaced it. Furthermore, it is a time and money-efficient technique that significantly increases sensitivity from 70% to 100% and is characterized by high throughput and acceptable phenotyping. When diagnosing HS is challenging, particularly when there are moderate clinical signs present, the final step suggests using geniting testing in conjunction with other screening tests (40). This is utilized in particular guidelines to estimate the alternatives for therapy (splenectomy or not), the progression of the disease, and, most importantly, to provide genetic counseling for patients and their families (41).**Table 4** showed how several genetic analysis techniques might be used to diagnose RBC cytoskeleton problems based on new gene mutations.

Table 4. Methods of genetic diagnosis in RBC membrane disorders and novel mutations diagnosis.

Methods	Membrane Diseases	Genes	Novel Mutation Nucleotide	Ref.
qPCR, NGS	HHA	*SPTA1*	mRNA c.4295del p. L1432*	(5)
			c.1120C > T, p. R374*	
			c.2671C > T p. R891*	
WES, Sanger Sequencing	HS	*SPTB*	c.5692 C>T, and c.3823delG	(6)
		ANK1	c.1994 C>A	
NGS	HPP	*SPTB allele 1, 2*	Missense, p.F2014V, c.6040T>G	(7)
	HPP	*SPTA1*	Missense, p.R2141W, c.6421C>T	
	HE	*EPB41*	Nonsense, c.784C>T, p.R262*	
WGS	HE	*SPTA1*	c.7220_7221del: p. Tyr 2407*	(8)
SDS-PAGE	HS	*ANK1*	p. A1867V	(9)
WGS	HE	*SPTB*	c.2303G>A pG768D	(10)
NGS	HS	*SPTB*	β-spectrin.	(11)
NGS	HS	*SPTB*	(NM_001355436.2: c.1645-1G>A	(12)
NGS	HS	*ANK1*	c.1638 C>A, p. Tyr546*	(13)
WGS	HS type3	SPTA1	c. 82C>T p. R28C chr1: 158655080	(14).
	HE type2			
WGS	HPP	*SPTA1*	c.5476C>T, pQ1826X	(15)
		SPTB	c.1041C>A, p.Y347X	
		SPTB	c.6224A>G, E2075G	
NGS	HE	*SPTA1*	exon2, c.86A > C, p. Gln29Prol.	(16)

NGS; Next Generation Sequencing, HHA; Hereditary Hemolytic Anemia, qPCR; Quantitative Real-Time PCR, WES; Whole-Exome Sequencing, HE; Hereditary Elliptocytosis, HPP; Hereditary Pyro Poikilocytosis, t-NGS; Targeted Next-Generation Sequencing, SDS-PAGE; Sulphate-Polyacrylamide Gel, Hereditary Elliptocytosis, hereditary spherocytosis, Electrophoresis.

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Results and discussion

The hereditary hemolytic disorders of HE, HPP, and HS are caused by mutations in the genes SPTA1 and SPTB, which encode spectrin alpha- and beta-chains, respectively (4). The main cause of anomalies in the membrane components of HS patients is gene mutations, which generally occur in an autosomal dominant pattern. The HS heterogeneity is caused by mutations in the genes SPTB, EPB42, ANK1, SPTA1, and SLC4A1, and the kind and frequency of these mutations differ by nation (7). Biallelic mutations in the genes encoding the cytoskeletal proteins protein 4.1R (EPB41), b-spectrin (SPTB), or a-spectrin (SPTA1) are seen in patients with HPP. Additionally, a diverse (severe (18)) genetic disorder of the cytoskeletal proteins and red blood cell membrane is the cause of hemolytic anemia. The SLC4A1 mutation Arg730Cys has been linked to patients with milder forms of Overhydrated Hereditary Stomatocytosis (OHS), while PIEZO1 indicates congenital lymphatic dysplasia with the description of loss of function mutations. Additionally, increased ABCB6 expression correlates with increased drug resistance in multiple cell lines, and KCNN4 is thought to be a potential target for therapeutic approaches in various diseases such as asthma, sickle cell anemia, atherosclerosis, kidney fibrosis, and autoimmunity. The majority of the mutations are found close to the protein's transport location. Furthermore, a particular band 3 mutation, Gly796Arg, which causes band 3 to convert from an anion exchanger to a cation transporter, has been linked to a case of DHS with dyserythropoietic, and the SLC2A1 This RBC cation content deficiency is caused by three distinct mutations: a loss of 12 nucleotides that results in Gln282_Ser285del in the transporter's seventh membrane-spanning helix, a substitution of Gly286Asp in the same helix, and a deletion of 3 nucleotides that results in Ile436del (24). Additionally, certain case reports have been published on mutations in these genes that have been linked to novel disorders such as vitamin B12(42), and hydrops fetalis (37) and immunodeficiency

(31) Mutations in the ANK1, SPTA1, SPTB, EPB42, and SLC4A1 genes cause the HS heterogeneity, and the prevalence and kind of these mutations vary by country (7). Furthermore, different regions and populations have different kinds of membrane protein abnormalities in HS, making diagnosis challenging (15). In conclusion, hemolytic syndromes and other organic disorders that are connected with one another are caused by mutations in proteins of the RBC cytoskeleton of membranes.

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