LABORATOIRE HOSPITALIER UNIVERSITAIRE DE BRUXELLES
LHUB-ULB
UNIVERSITAIR LABORATORIUM BRUSSEL

Hereditary Red Cell Membrane Disorders

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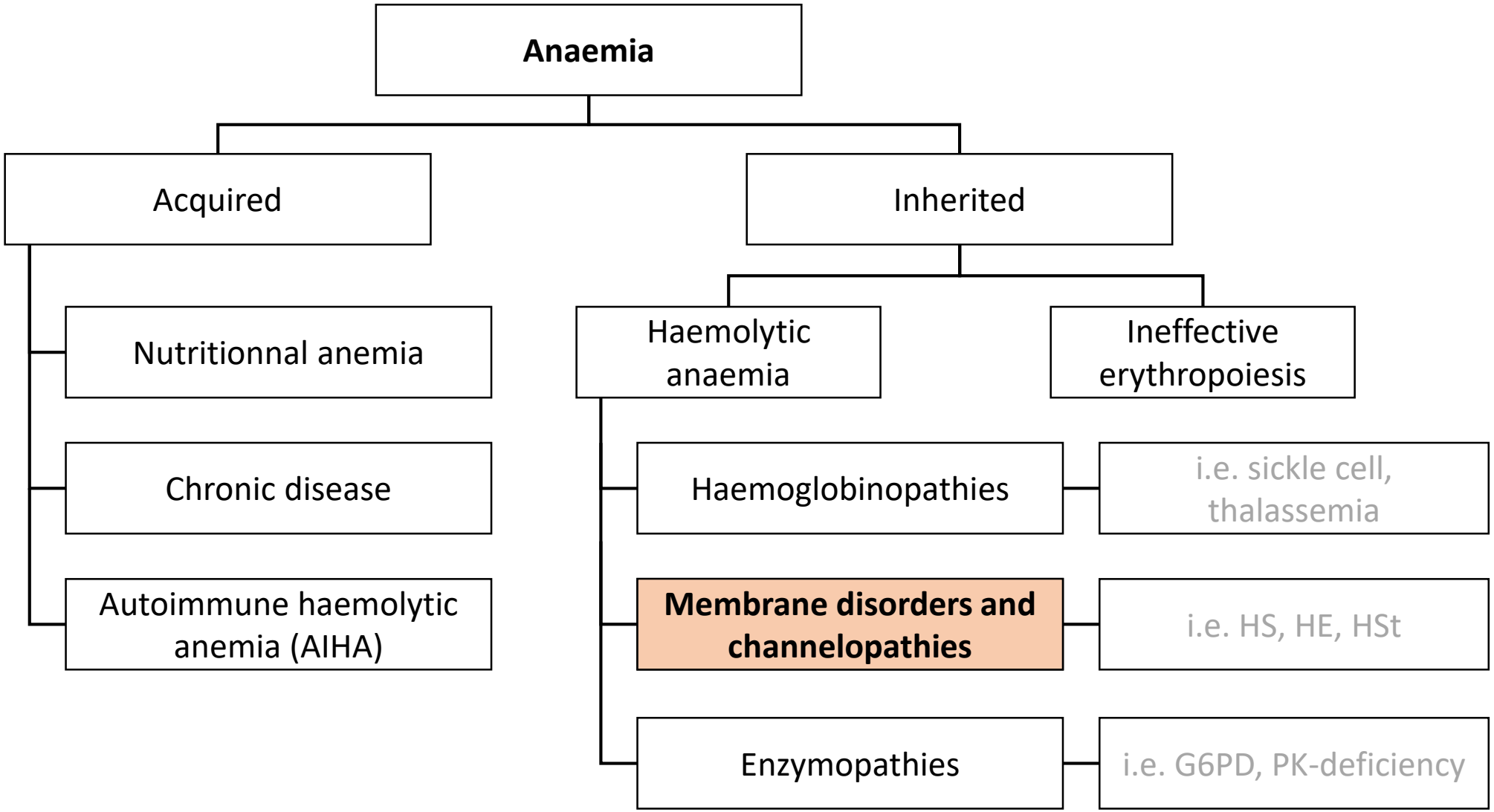
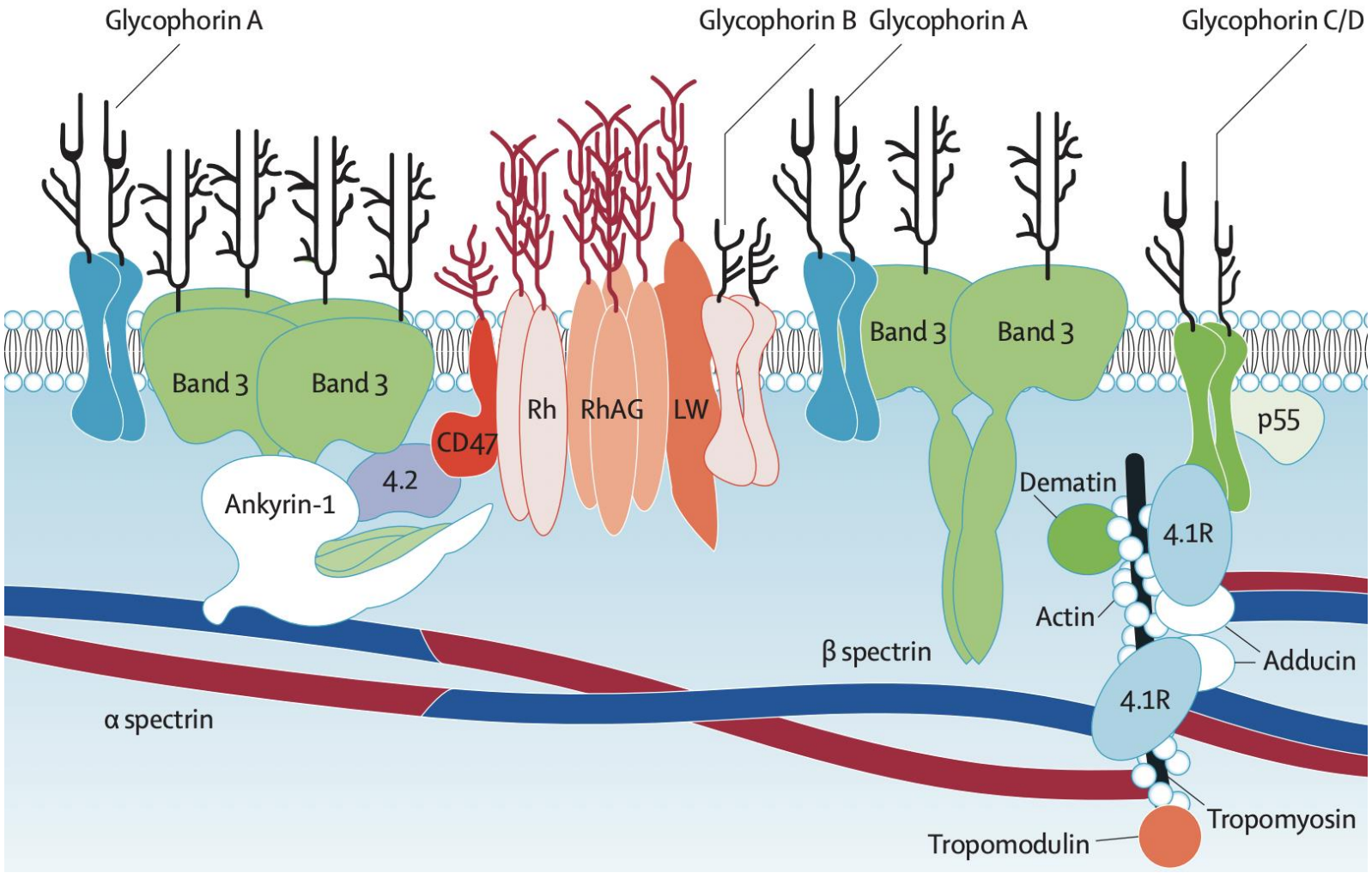
Manama Course in Clinical Genetics – 18/04/2023

INTRODUCTION

Anaemia

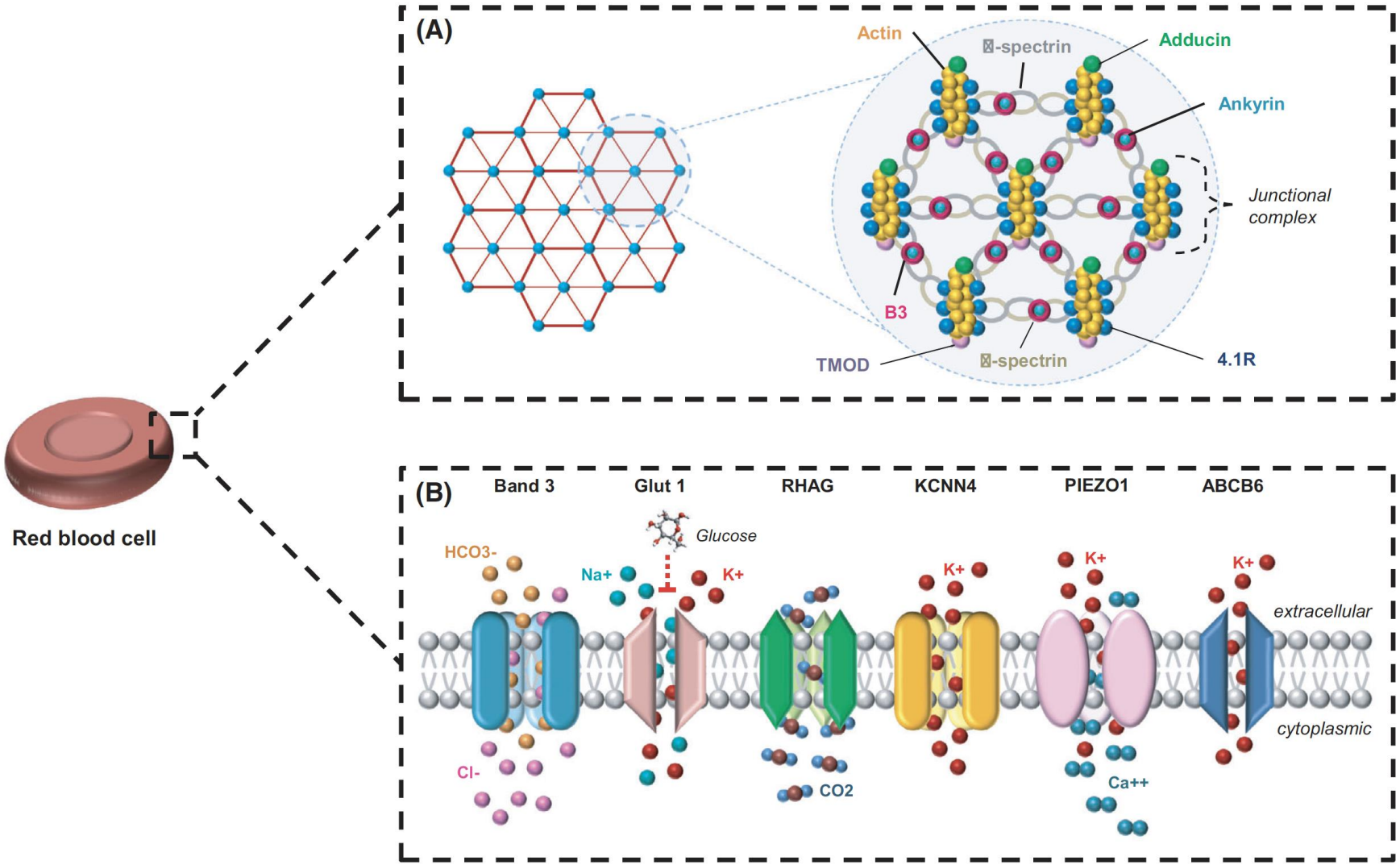

Anaemia
Acquired
Inherited
Nutritionnal anemia
Chronic disease
Autoimmune haemolytic anemia (AIHA)
Haemolytic anaemia
Ineffective erythropoiesis
Haemoglobinopathies
i.e. sickle cell, thalassemia
Membrane disorders and channelopathies
i.e. HS, HE, HSt
Enzymopathies
i.e. G6PD, PK-deficiency

Red Cell Membrane



Perrotta S, A et al. 2018

Red Cell Membrane



Iolascon, A et al. 2019

Hereditary Red Cell Membrane Disorders

Structural Disorders

Hereditary Spherocytosis (HS)

Hereditary Elliptocytosis (HE)

Hereditary Pyropoikilocysosis (HPP)

Membrane Transport Disorders

Overhydrated Hereditary Stomatocytosis (OHSt)

Dehydrated Hereditary Stomatocytosis (DHSt)*

Familial Pseudohyperkalaemia (FP)

Cryohydrocytosis (CHC)

Southeast Asian Ovalocytosis (SAO)

*Hereditary Xerocytosis (HX)

HEREDITARY SPHEROCYTOSIS

Hereditary Spherocytosis

- First described in **1871** as **microcythemia** in a case history by 2 Belgian physicians

- **Most common inherited haemolytic anemia:**
  - **Prevalence:** 1/2.000 – 1/5.000 in Nothern Europe
  - Probably **higher** (undiagnosed mild cases)

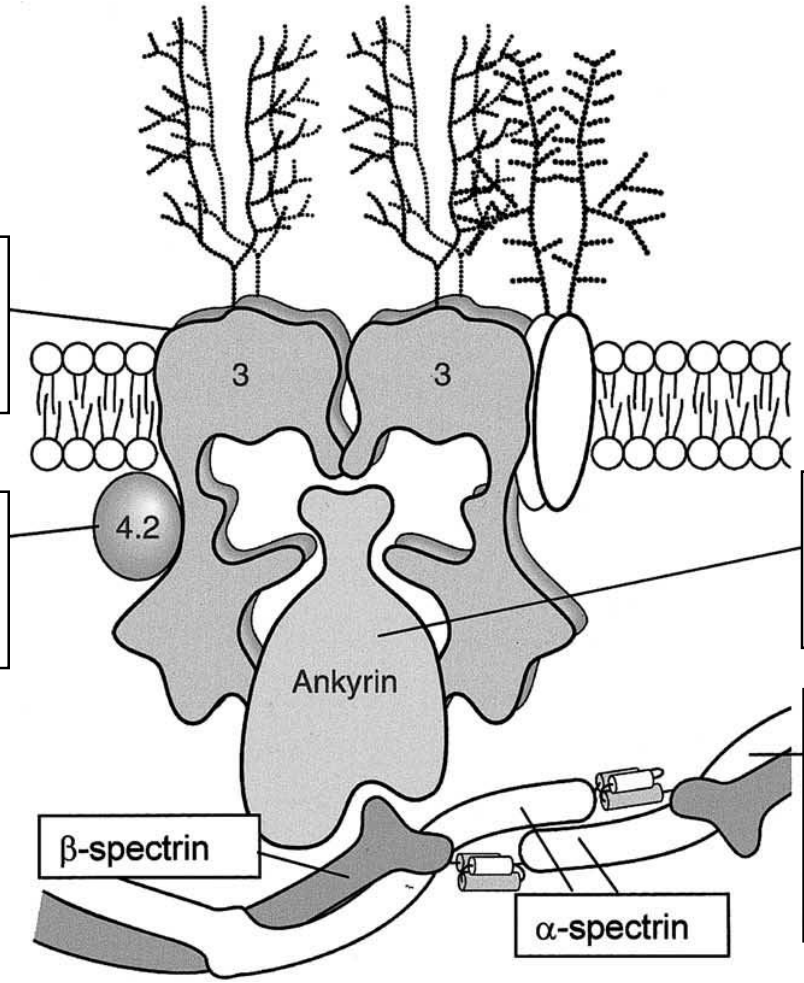
- **Highly heterogeneous group of disorders:**
  - **Clinical severity:** fully compensated haemolysis to transfusion-dependant anaemia
  - **Protein defect:** α- and β-spectrins, ankyrin, band 3 and protein 4.2
  - **Mode of inheritance:** 75% dominant ; 25% recessive/*de novo*
  - **Age of diagnosis**

Hereditary Spherocytosis

Protein	Gene	Chromosome localisation
α-spectrin	SPTA1	1q22-q23
β-spectrin	SPTB	14q23-q24.2
Ankyrin	ANK1	8p11.2
Band 3	EPB3	17q12-q21
4.2 protein	ELP42	15q15-q21

Mutations of **Band 3** in **20-35%** of HS – mostly **dominant**

Mutations of **Protein 4.2** in **recessive** HS (**<5%** of HS)



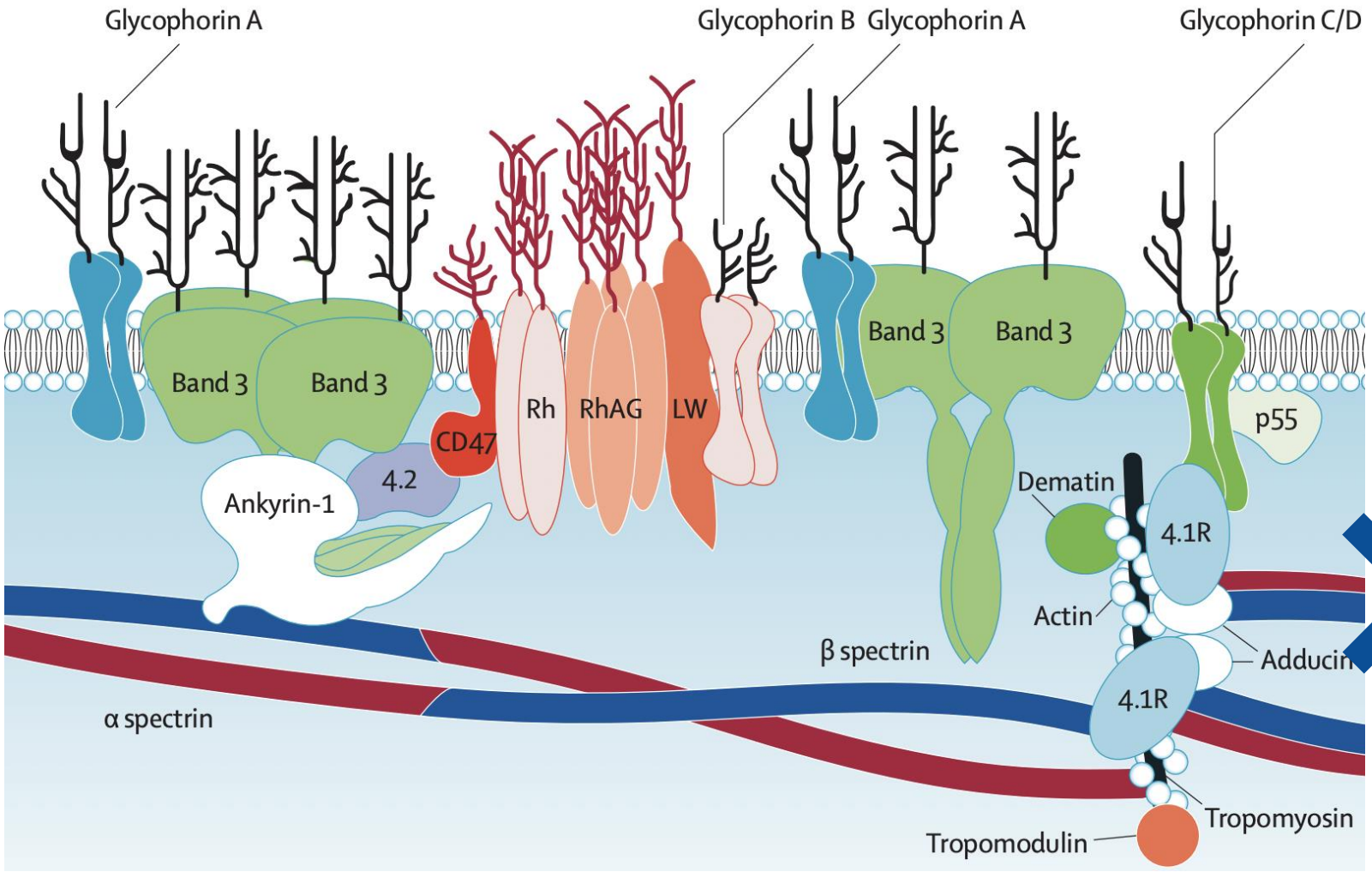
Mutations of **Ankyrin** in **40-65%** of HS – both **dominant** and **recessive**

Mutations of **α-spectrin** in **recessive** HS (**<5%** of HS)

Mutations of **β-spectrin** in **dominant** HS (**15-30%** of HS)

Adapted from Eber et al. 2004 and Perrotta S, A et al. 2018

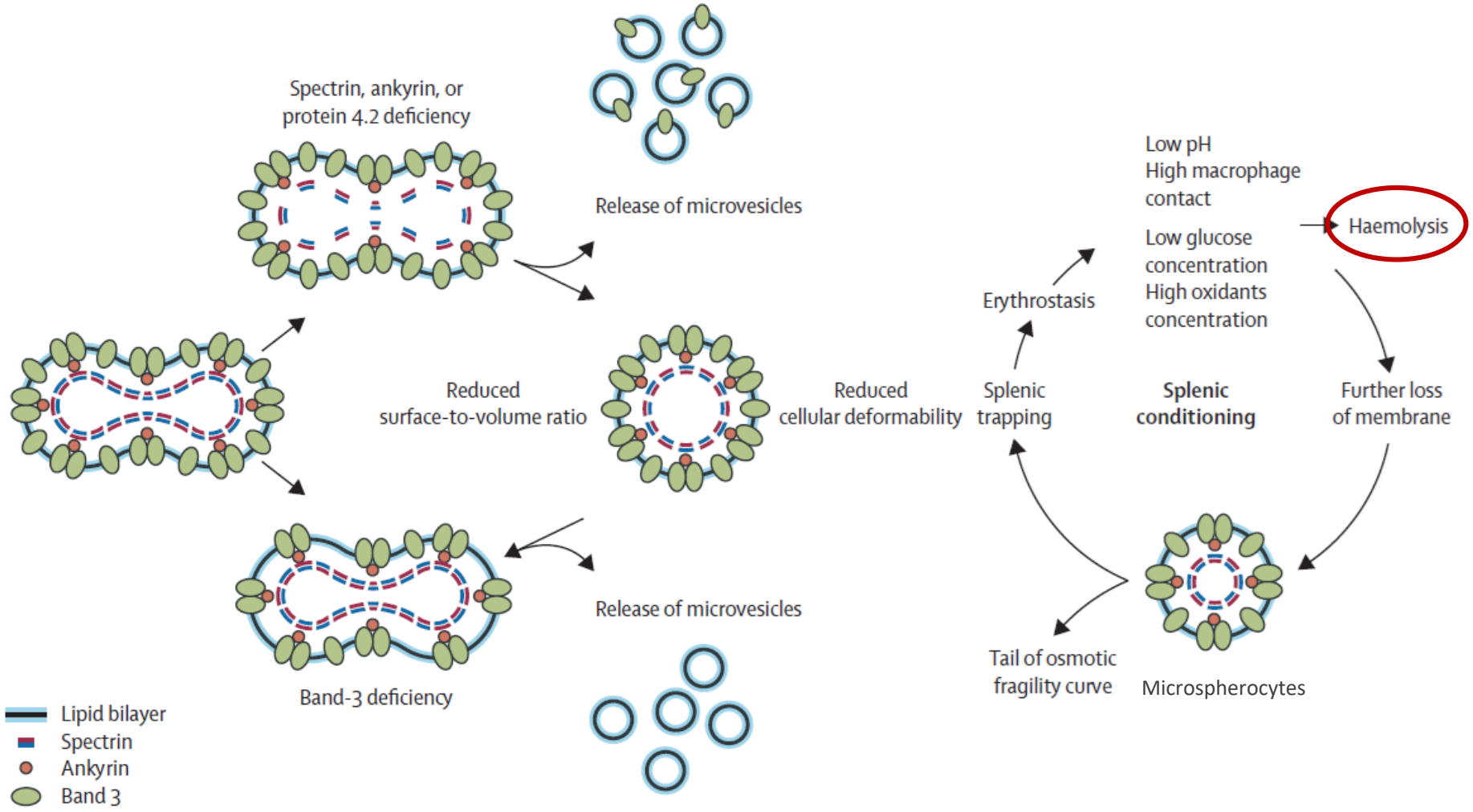
Hereditary Spherocytosis



⇨ **Membrane loss (µvesicles)**

Perrotta S, A et al. 2018

Hereditary Spherocytosis



Perrotta S, A et al. 2018

Hereditary Spherocytosis

- **Clinical presentation:**
  - **Neonatal period/Infancy:**
    - Neonatal jaundice
    - Hb level:
      - Normal at birth
      - Rapid fall within the 1st month after birth
    - Anaemia: mostly improves during the 1st year of life
  - **Childhood/Adulthood:**
    - Persistant jaundice, anaemia, splenomegaly, gallstones
    - Haemolysis: can be compensated in adults
- **Positive familial history** in 75% of cases

Hereditary Spherocytosis

	Trait	Mild	Moderate	Severe
Haemoglobin (g/dL)	Normal	11 – 15	8 – 12	6 – 8
Reticulocytes count (%)	Normal (< 3%)	3 – 6	> 6	> 10
Bilirubin (µmol/L)	< 17	17 – 34	> 34	> 51
Splenectomy	Not required	Usually not necessary during childhood and adolescence	Necessary during school age before puberty	Necessary – delay until 6 years if possible

HEREDITARY ELLIPTOCYTOSIS

Hereditary Elliptocytosis

- **Heterogeneous group** of inherited RBC membrane disorders characterised by **elliptical-shaped RBCs** on the peripheral blood smear

- **Subtypes of HE:**
  - Common Hereditary elliptocytosis (HE)
  - Hereditary PyroPoikilocytosis (HPP)
  - Southeast Asian Ovalocytosis (SAO)
  - Spherocytic Elliptocytosis (SE)

⇨ Major clinical differences in the **RBC morphology** and **severity of haemolysis**

Hereditary Elliptocytosis

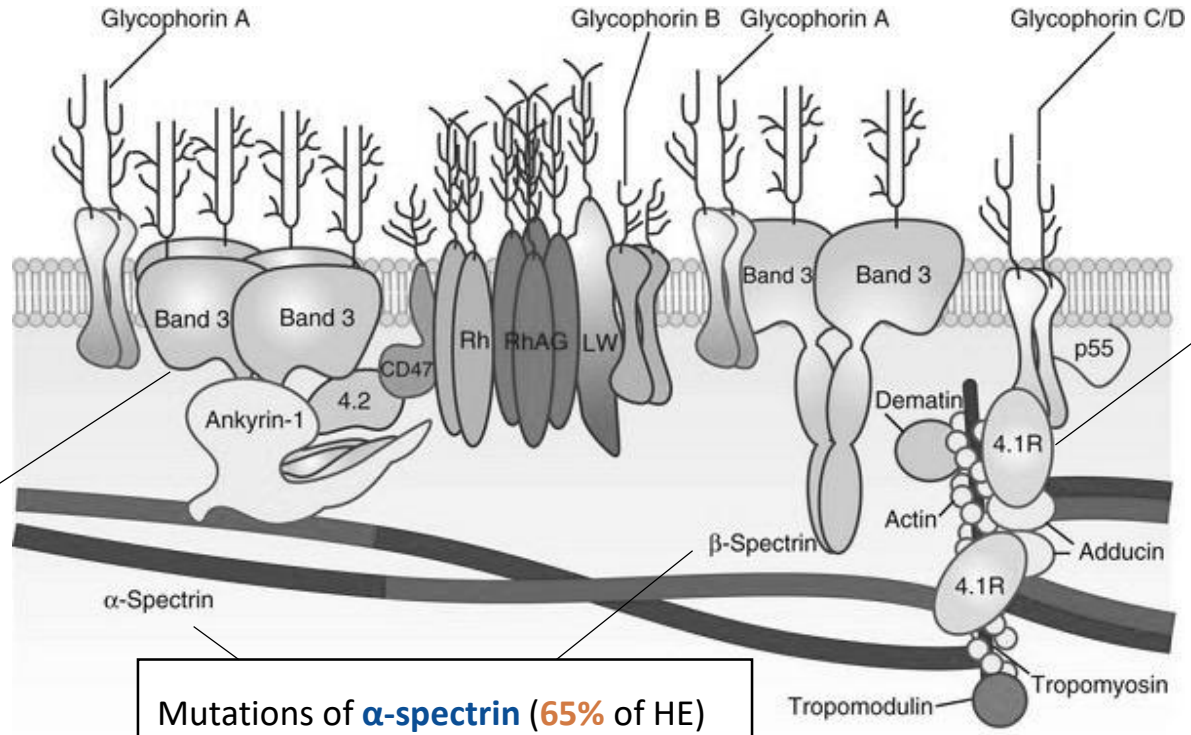
- First reported in **1904** by Dresbach

- **Prevalence:** Unknown
  - Estimated to 1/1.000 – 1/4.000 worldwide
    - 1/5.000 among Caucasians
    - Higher in countries in the malaria-endemic regions : 1/100 in West Africa (Probable protection)
  - Probably **higher** (undiagnosed asymptomatic patients)

- **Highly heterogeneous group of disorders:**
  - **Clinical severity:** asymptomatic to severe haemolytic anaemia
  - **Protein defect:** α and β-spectrins, protein 4.1 (and rarely glycophorin C) (Band 3 in SAO)
  - **Mode of inheritance:** autosomal dominant (recessive for HPP)
  - **Age of diagnosis**

Hereditary Elliptocytosis

Protein	Gene	Chromosome localisation
α-spectrin	SPTA1	1q22-q23
β-spectrin	SPTB	14q23-q24.2
4.1 protein	EBP41	¨1p33-34.2
Band 3	SLC4A1	17q12-q21



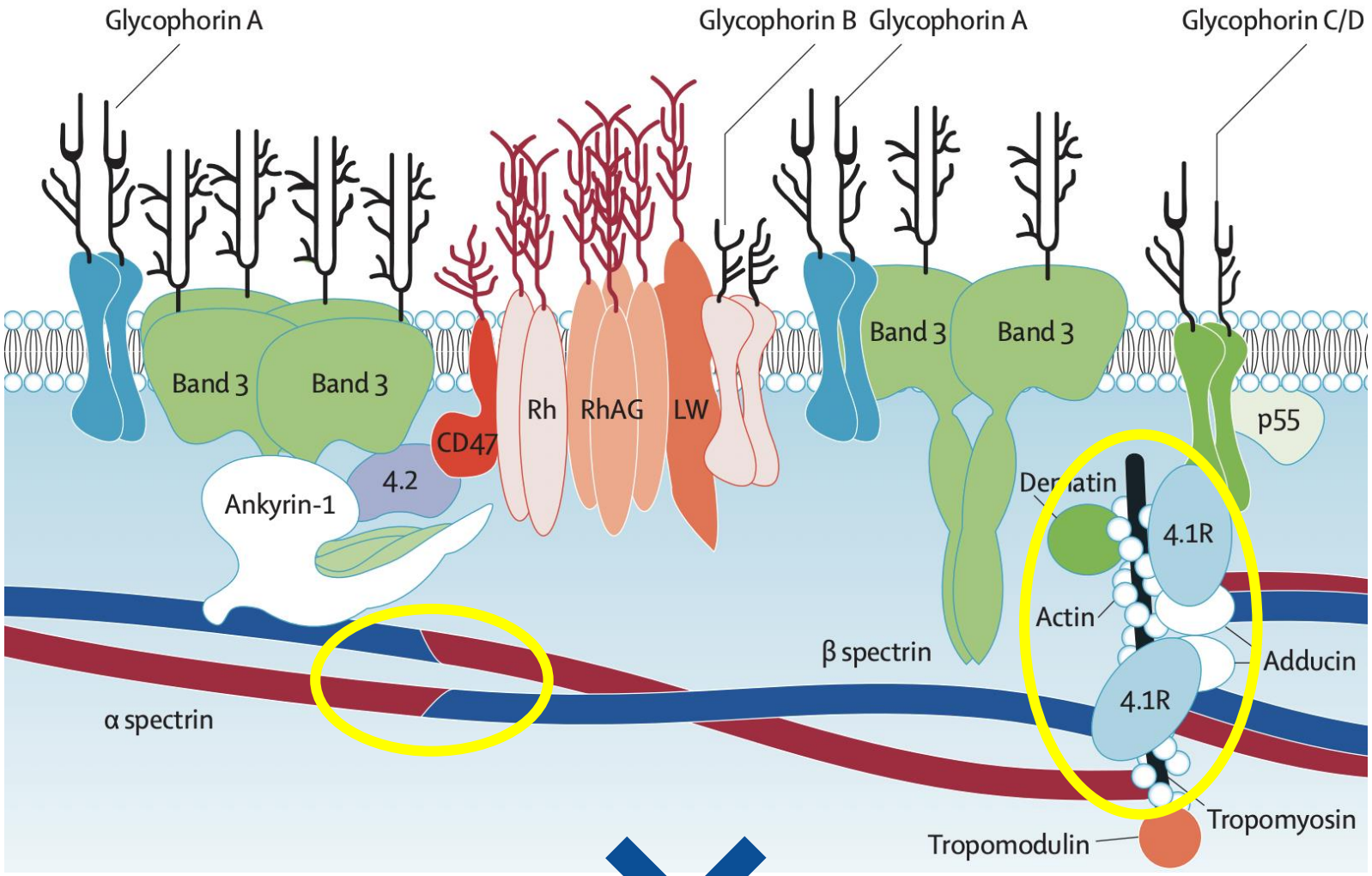
A **specific band 3 variant** causes SAO

Mutations of **protein 4.1** (**5%** of HE ; **30%** in Europe)

Mutations of **α-spectrin** (**65%** of HE)

Mutations of **β-spectrin** (**30%** of HE)

Hereditary Elliptocytosis



Perrotta S, A et al. 2018

Hereditary Elliptocytosis

- **Physiopathology:**
  - **Normal RBCs:** repeatedly and momentarily assume an elliptical shape to negotiate through capillaries but then regain their biconcave discoid shape after they pass through the microcirculation
  - **RBCs in HE:**
    - Lack the elastic recoil necessary for returning to the discoid shape and eventually assume the fixed characteristic morphology of elliptocytes
    - Elliptocytes are not as deformable as normal RBCs and are eventually trapped and removed by the spleen
  - In severe case (HPP), **membrane is lost**, leading to fragmentation, haemolysis and production of microcytic or spherotic RBCs

Hereditary Elliptocytosis

	Common HE	HPP	SAO	Spherocytic Elliptocytosis
Haemolytic Anemia	None-mild	Severe	None (Possible in neonates)	Mild to moderate
Spenomegaly	None	Present	None	Present
Other clinical manifestations	None	Intermittent jaundice Aplastic crises	None	Intermittent jaundice Aplastic crises
Peripheral blood smear	15-90% elliptocytes	Poikilocytosis; RBC budding with fragments; elliptocytes; microspherocytes	Rounded elliptocytes, some having a transverse bar dividing cell	Rounded elliptocytes; spherocytes
Inheritance	Dominant	Recessive	Dominant	Dominant

HEREDITARY STOMATOCYTOSIS

Hereditary Stomatocytosis

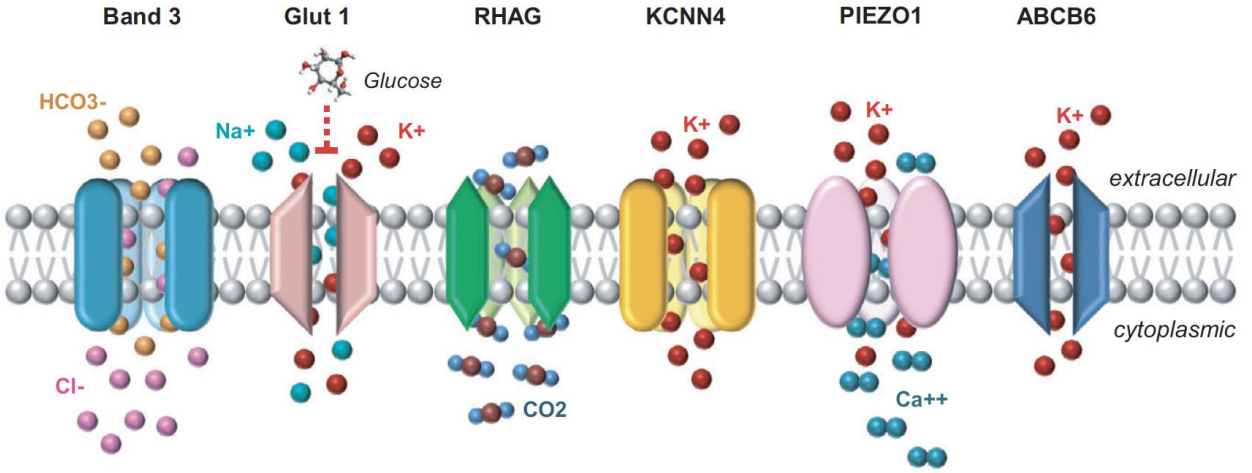
› Blood. 1971 Aug;38(2):184-204.

A new variant of hereditary hemolytic anemia with stomatocytosis and erythrocyte cation abnormality

D R Miller, F R Rickles, M A Lichtman, P L La Celle, J Bates, R I Weed

- Group of haemolytic conditions in which the primary lesion is a **« leak » to the monovalent cations Na^+ and K^+**, resulting in an **altered hydratation status** shown by a significant change in MCV

- **Subtypes of HSt:**
  - Overhydrated HSt (OHSt)
  - Dehydrated HSt (DHSt)
  - Cryohydrocytosis (CHC)
  - Familial pseudohyperkalaemia (FP)



- **Heterogeneous group of disorders:**
  - **Clinical severity:** highly variable clinical presentation**;** only FP is an asymptomatic trait
  - **Protein defect:** PIEZO1 channel, Gardos channel (KCNN4), RhAG, Band 3 and ABCB6
  - **Mode of inheritance:** dominant
  - **Age of diagnosis**

Hereditary Stomatocytosis

	Overhydrated HSt (OHSt)	Dehydrated HSt (DHSt)	Cryohydrocytosis (CHC)	Familial Pseudo-hyperkalaemia (FP)
Prevalence	1/1.000.000	1/10.000	Rare	Rare
Morphology	Macrocytosis, stomatocytosis	Stomatocytes, Target cells	Stomatocytes	Normal
Hb (g/dL)	8-10	12-15	10-12	Normal range
MCV (fL)	120-140	120-150	100-120	Normal or high
MCHC (g/dL)	24-28	35-37	Normal	Normal
Reticulocytosis	10-15%	About 10%	About 8%	Normal
Haptoglobin	Undetectable	Undetectable	Undetectable	Present
Intracellular cations	About 40x normal Na^+/K^+ transport rate	Abnormal, more subtle than OHSt	K^+ leak at low temperature and at 4°C	High plasma K^+ when blood specimen left at RT for several hours

Adapted from King et al., 2015

Hereditary Stomatocytosis

- **Physiopathology:**
  - Precise mechanisms leading to RBCs haemolysis **largely unknown**
  - Mechanism of stomatocyte formation involves **changes in cell volume** caused by **changes in intracellular ion content**
    - When leakage of K^+ out of the cell exceeds the rate at which it's pumped back in → Low intracellular K^+ content → **Dehydratation**
    - If the permeability of the membrane to Na^+ is abnormally high and the Na^+/K^+-ATPase is unable to fully compensate → Na^+ cell gain → **Overhydratation**

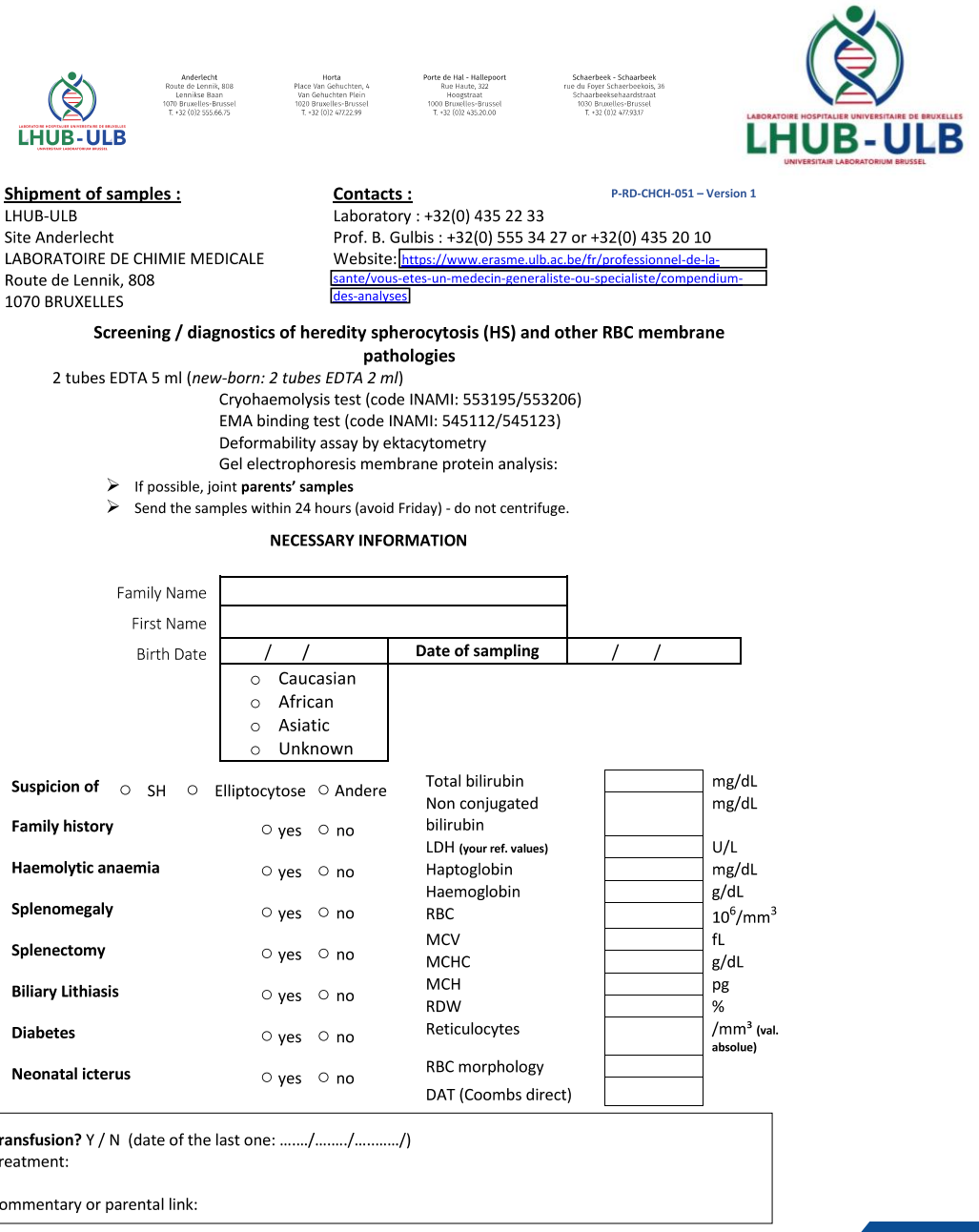
DIAGNOSIS

PHENOTYPE

LHUB-ULB Laboratory approach

- **Family and Clinical Histories**

- **Laboratory investigations**
  - **First tier screening tests**
    - RBC morphology
    - Biological parameters of haemolysis
    - (New RBC and reticulocyte parameters)
  - **Second tier screening tests**
    - Eosine-5-maleimide (EMA) binding test
    - Cryohaemolysis
  - **Diagnostic tests**
    - Ektacytometry
    - SDS-PAGE



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P-RD-CHCH-051 – Version 1

Screening / diagnostics of heredity spherocytosis (HS) and other RBC membrane pathologies

2 tubes EDTA 5 ml (*new-born: 2 tubes EDTA 2 ml*)
Cryohaemolysis test (code INAMI: 553195/553206)
EMA binding test (code INAMI: 545112/545123)
Deformability assay by ektacytometry
Gel electrophoresis membrane protein analysis:

- If possible, joint **parents' samples**
- Send the samples within 24 hours (avoid Friday) - do not centrifuge.

NECESSARY INFORMATION

Family Name			
First Name			
Birth Date	/ /	**Date of sampling**	/ /

○ Caucasian ○ African ○ Asiatic ○ Unknown

Suspicion of ○ SH ○ Elliptocytose ○ Andere

Family history	○ yes	○ no	Total bilirubin	mg/dL
Haemolytic anaemia	○ yes	○ no	Non conjugated bilirubin	mg/dL
Splenomegaly	○ yes	○ no	LDH **(your ref. values)**	U/L
Splenectomy	○ yes	○ no	Haptoglobin	mg/dL
Biliary Lithiasis	○ yes	○ no	Haemoglobin	g/dL
Diabetes	○ yes	○ no	RBC	$10^6/mm^3$
Neonatal icterus	○ yes	○ no	MCV	fL
			MCHC	g/dL
			MCH	pg
			RDW	%
			Reticulocytes	/mm^3 **(val. absolue)**
			RBC morphology	
			DAT (Coombs direct)	

Transfusion? Y / N (date of the last one: ...../...../...../)
Treatment:

Commentary or parental link:

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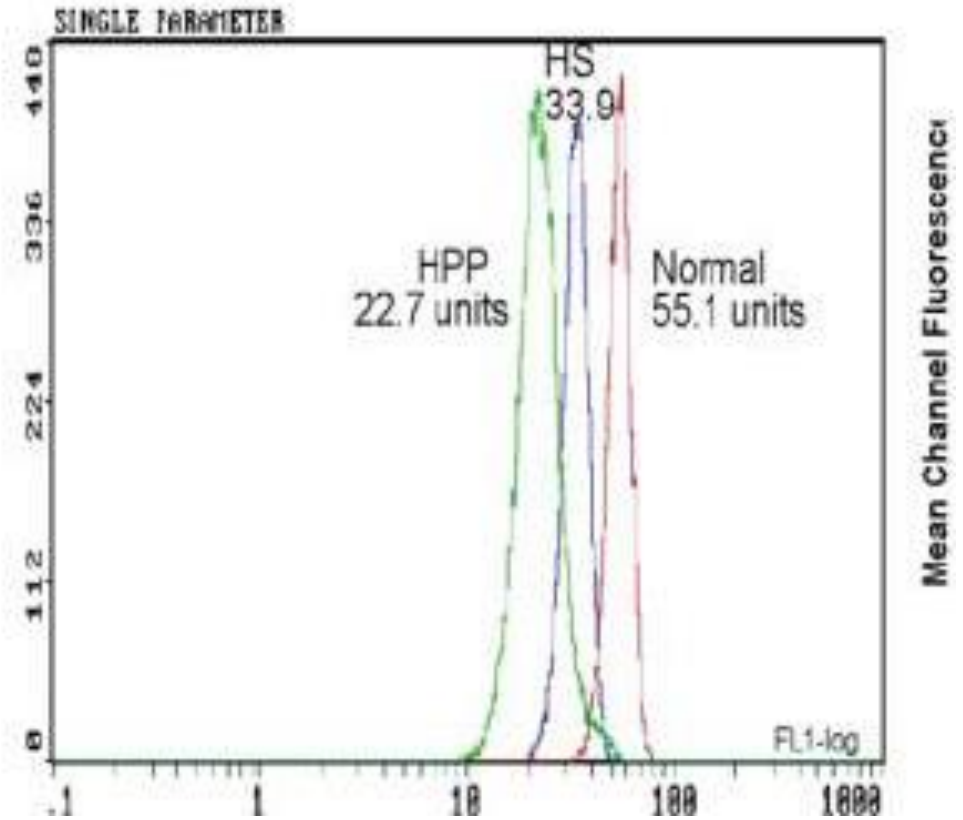
Cryohaemolysis test

- Test based on the **increased susceptibility to cold (0°c) in hypertonic conditions** of the RBCs of HS patients
  - Measure the **% of cryohaemolysis** compared to a normal control
  - **Cut-off value:** positive if > 10%
  - **Performance**:
    - Sensitivity: 100%
    - Specificity: 86%

EMA Binding Test

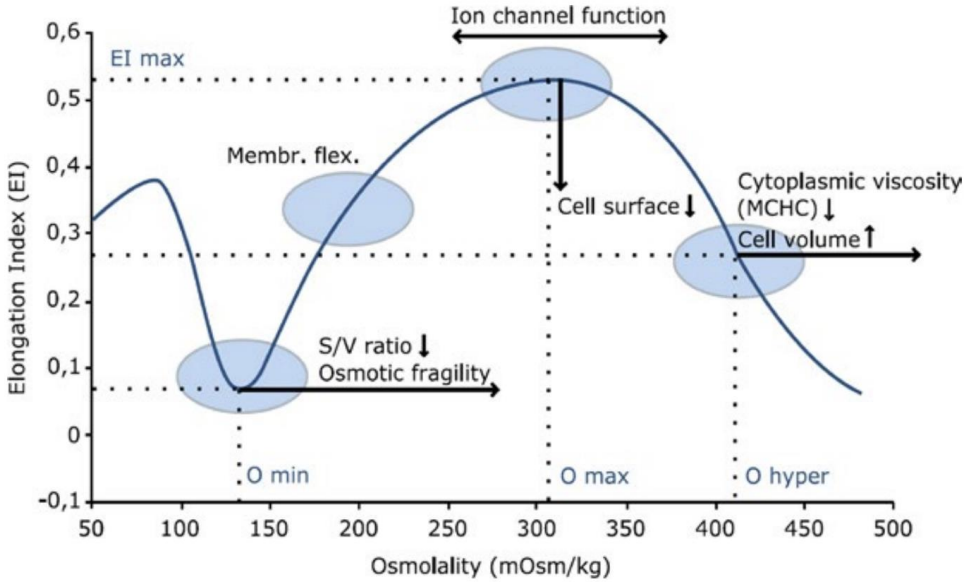
- Flow cytometric test that measures the **mean fluorescence intensity (MFI) of EMA tagged RBCs**
  - EMA dye binds mostly to **Band 3 protein**
  - **Results:** expressed in % decrease in MFI of patients compared to the mean of normal controls
  - **Cut-off values:**
    - < 11%: negative
    - 11-19%: grey zone
    - > 19%: positive
  - **Performances**:
    - Sensitivity: 89 - 99,1%
    - Specificity: 92,7 - 99,1%
  - **Advantages:** Simple, Cost effective & Highly reproductible

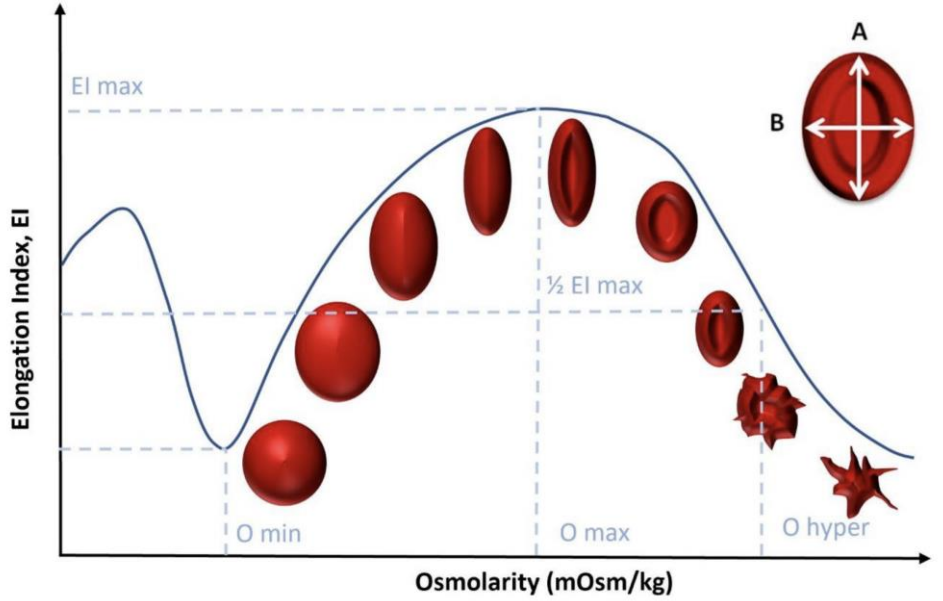


Ektacytometry

- **Ektacytometer:**
  - **Fully automated measurement and calculation** of various phenomena of RBCs by analysis of their rheological behavior
  - **Accurate detection** of :
    - RBC deformability
    - Pre-hemolytic stability
    - RBC aggregation

- **Osmoscan:** analysis of the **RBC deformability** in **changing osmotic environment** with applied constant shear stress

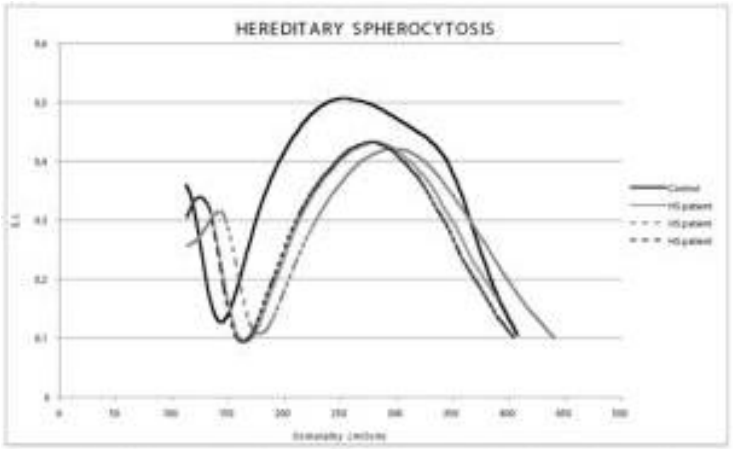




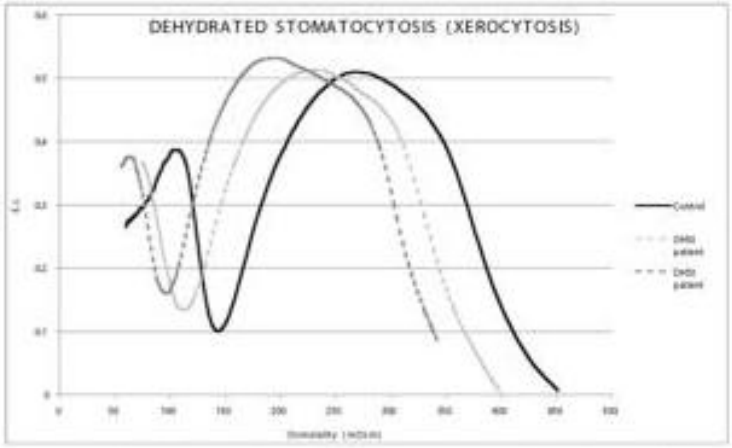
Méndez-Mora L *et al*, 2021

Ektacytometry

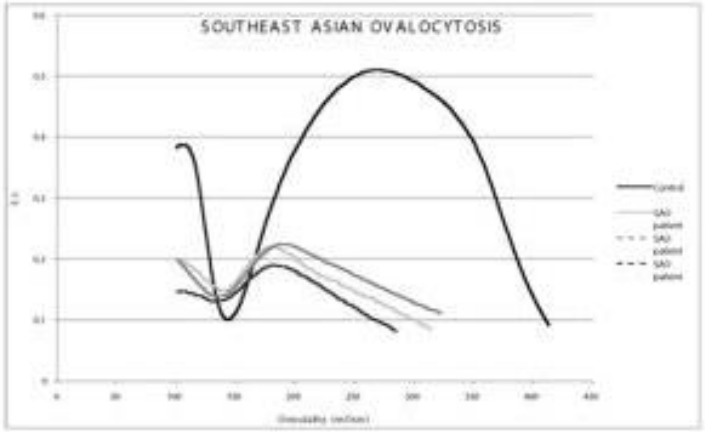
Hereditary Spherocytosis



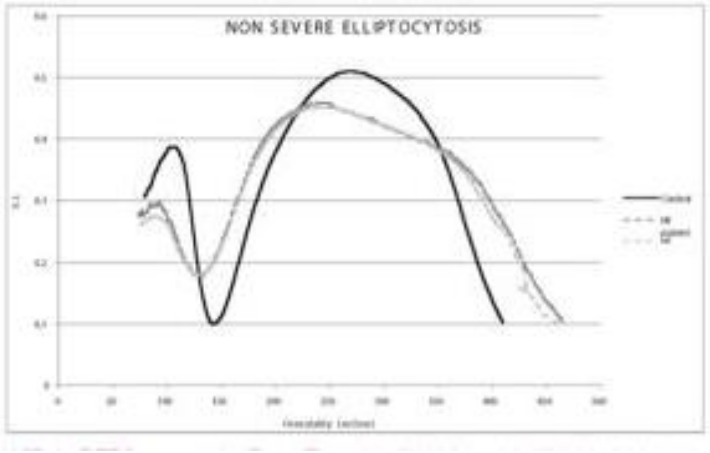
Dehydrated Hereditary Stomatocysosis



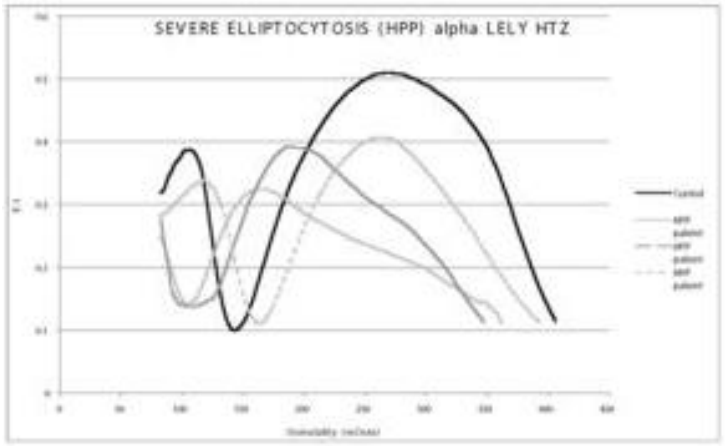
Southeast Asian Ovalocytosis



Hereditary Elliptocytosis



Hereditary Pyropoikilocytosis



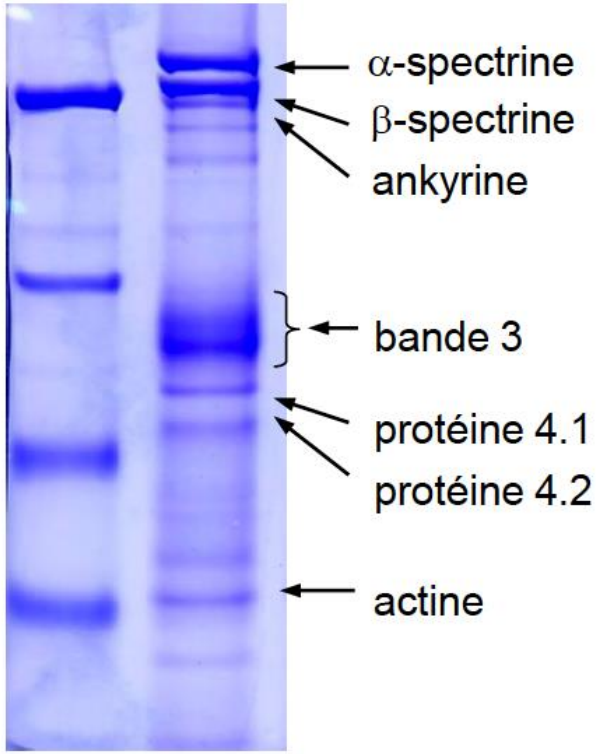
Da Costa et al, 2015

SDS-PAGE

- **SDS-PAGE =** Sodium dodecyl sulphate polyacrylamide gel electrophoresis
  - Determines the **defective membrane protein** and the **extent of membrane deficiency**
  - **Lack of sensitivity** to some of the mild HS

- **Recommended if:**
  - Clinical phenotype **more severe than predicted from RBC morphology**
  - RBC morphology is **more severe than predicted from parental blood film**
  - **Equivocal or borderline results** of the screening tests
  - Diagnosis is **not clear** prior to splenectomy

- **Can not detect GLUT1, RhA, Gardos G and PIZEO1 related disorders**



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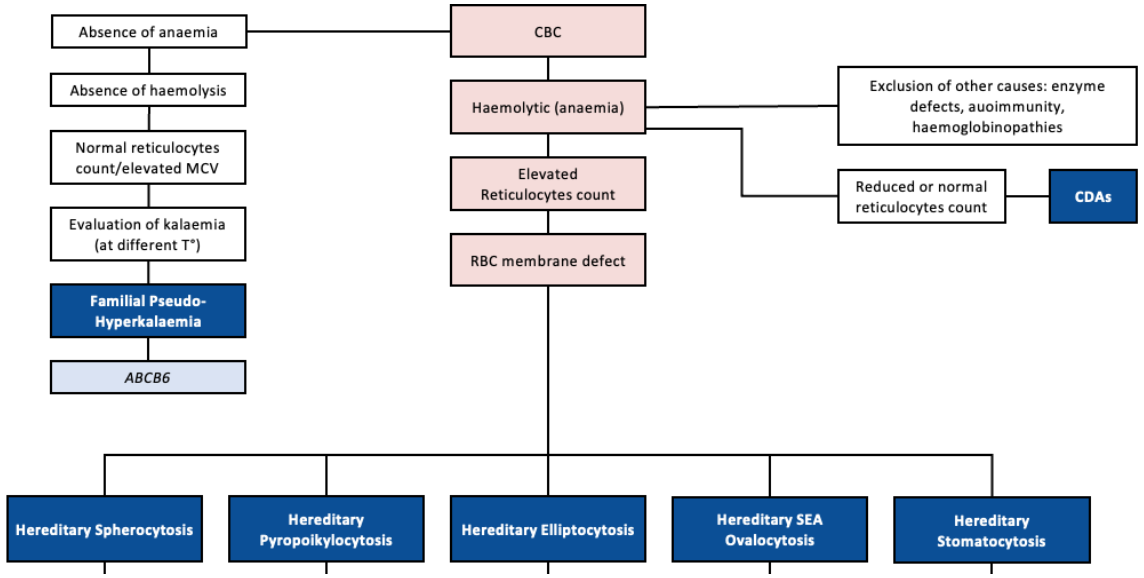
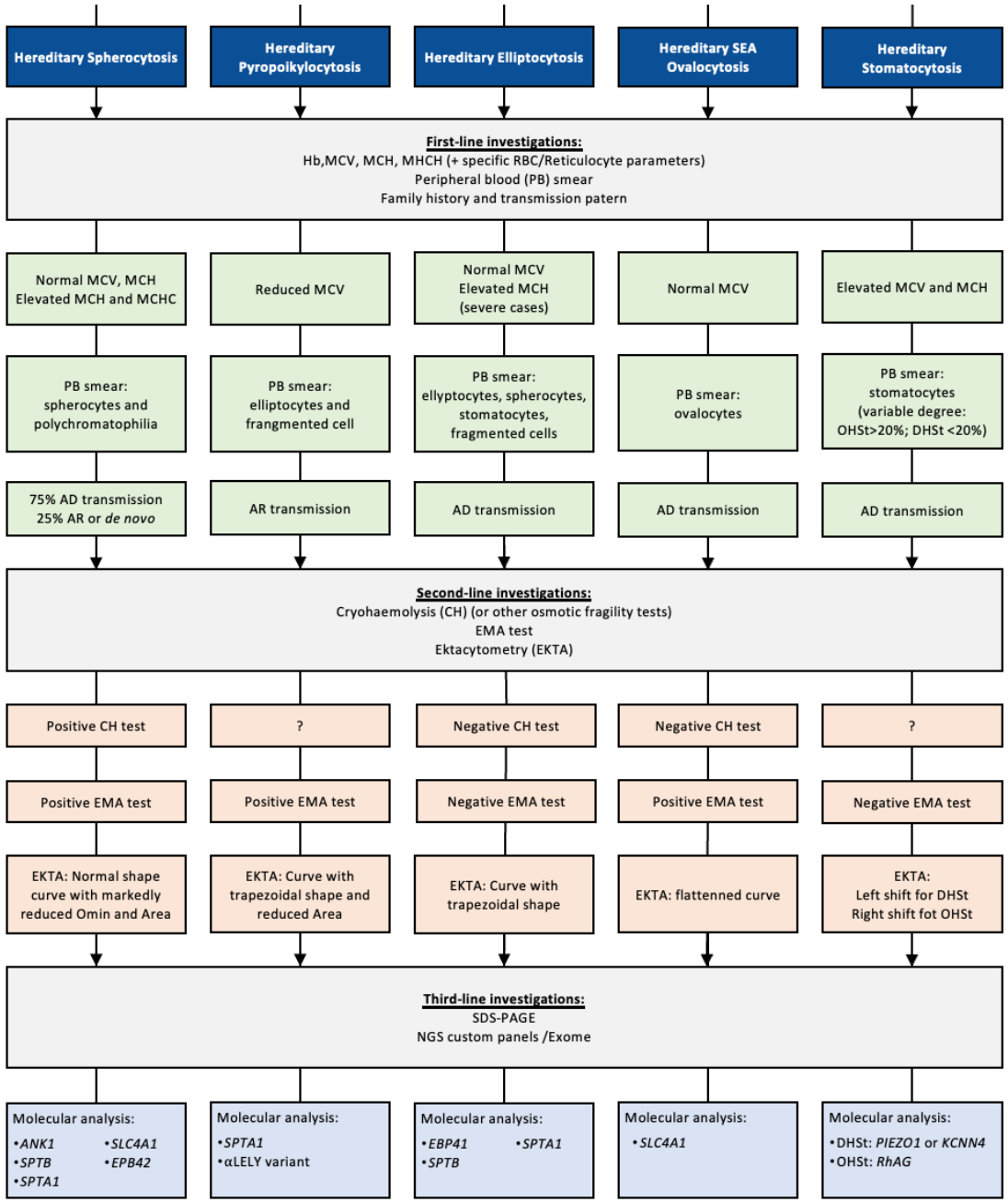
- **2019-Present:** National Reference Center for the analysis of
  - RBC deformability by **Ektacytometry**
  - RBC membrane proteins by **SDS-PAGE**

LHUB-ULB Flow Chart



Adapted from Andolfo I *et al.*, 2016



Adapted from Andolfo I *et al.*, 2016

GENOTYPE

OMIM

- **Genetic standpoint:** 15 different types of anaemias due to RBC membrane defects included in the Online Mendelian Inheritance in Man (OMIM) compendium of human genes and genetic phenotypes

Table 1. Classification of erythrocyte membrane disorders by OMIM database.

Disease symbol	Phenotype	Phenotype MIM number	Gene *location*	Protein name[§]	Inheritance
HS1	Hereditary spherocytosis type 1	182900	ANK1 *8p11.21*	Ankyrin-1	AD
HS2	Hereditary spherocytosis type 2	616649	SPTB *14q23.3*	Spectrin β chain, erythrocytic	AD
HS3	Hereditary spherocytosis type 3	270970	SPTA1 *1q23.1*	Spectrin α chain, erythrocytic 1	AR
HS4	Hereditary spherocytosis type 4	612653	SLC4A1 *17q21.31*	Band 3 anion transport protein	AD
HS5	Hereditary spherocytosis type 5	612690	EPB42 *15q15.2*	Erythrocyte membrane protein band 4.2	AR
HE1	Hereditary elliptocytosis 1	611804	EPB41 *1p35.3*	Protein band 4.1	AD
HE2	Hereditary elliptocytosis 2	130600	SPTA1 *1q23.1*	Spectrin α chain, erythrocytic 1	AD
HE3	Hereditary elliptocytosis 3	-	SPTB *14q23.3*	Spectrin β chain, erythrocytic	AD
HPP	Hereditary Pyropoikilocytosis	266140	SPTA1 *1q23.1*	Spectrin α chain, erythrocytic 1	AR
SAO	Ovalocytosis Southeast Asian type	166900	SLC4A1 *17q21.31*	Band 3 anion transport protein	AD
OHS	Overhydrated hereditary stomatocytosis	185000	RHAG *6p12.3*	Ammonium transporter Rh type A	AD
DHS1	Dehydrated hereditary stomatocytosis with or without pseudohyperkalemia and/or perinatal edema	194380	PIEZO1 *16q24.3*	Piezo-type mechanosensitive ion channel component 1	AD
DHS2	Dehydrated hereditary stomatocytosis 2	616689	KCNN4 *19q13.31*	Intermediate conductance calcium-activated potassium channel protein 4	AD
FP	Familial pseudohyperkalemia	609153	ABCB6 *2q35-q36*	ATP-binding cassette sub-family B member 6	AD
CHC	Cryohydrocytosis	185020	SLC4A1 *17q21.31*	Band 3 anion transport protein	AD

[§]Protein name reported in Uniprot database. AD: Autosomal dominant; AR: Autosomal recessive; ATP: adenosine triphosphate; Rh: Rhesus; OMIM: Online Mendelian Inheritance in Man; MIM: Mendelian Inheritance in Man.

Andolfo I *et al.*, 2016

Molecular analysis: When?

Clinical Phenotype / Laboratory testing

- Transfusion-dependent patients
- Recessive or sporadic transmission
- Complex forms of membrane pathology or mixed forms
- Discrepancy between clinical phenotype and diagnosis
- Cases of unexplained fetal anaemia or hydrops fetalis
- Unsolved cases

Next Generation Sequencing (NGS)

Molecular analysis: Why?

Multi-gene panel testing improves diagnosis and management of patients with hereditary anemias

Roberta Russo, Immacolata Andolfo, Francesco Manna, Antonella Gambale, Roberta Marra, Barbara Eleni Rosato, Paola Caforio, Valeria Pinto, Piero Pignataro, Kottayam Radhakrishnan, Sule Unal, Giovanna Tomaiuolo, Gian Luca Forni, Achille Iolascon

Am J Hematol. 2018 May;93(5):672-682.

A novel 33-Gene targeted resequencing panel provides accurate, clinical-grade diagnosis and improves patient management for rare inherited anaemias

Noémi B. A. Roy, Edward A. Wilson, Shirley Henderson, Katherine Wray, Christian Babbs, Steven Okoli, Wale Atoyebi, Avery Mixon, Mary R. Cahill, Peter Carey, Jonathan Cullis, Julie Curtin, Helene Dreau, David J. P. Ferguson, Brenda Gibson, Georgina Hall, Joanne Mason, Mary Morgan, Melanie Proven, Amrana Qureshi, Joaquin Sanchez Garcia, Nongnuch Sirachainan, Juliana Teo, Ulf Tedgård, Doug Higgs, David Roberts, Irene Roberts, Anna Schuh **... See fewer authors**

Br J Haematol. 2016 Oct;175(2):318-330

Detection of new pathogenic mutations in patients with congenital haemolytic anaemia using next-generation sequencing

R. Del Orbe Barreto, B. Arrizabalaga, A. B. De la Hoz, Á. García-Orad, M. I. Tejada, J. C. Garcia-Ruiz, T. Fidalgo, C. Bento, L. Manco, M. L. Ribeiro

Int J Lab Hematol. 2016 Dec;38(6):629-638.

Clinical utility of next-generation sequencing in the diagnosis of hereditary haemolytic anaemias

Archana M. Agarwal, Roberto H. Nussenzveig, Noel S. Reading, Jay L. Patel, Nikhil Sangle, Mohamed E. Salama, Josef T. Prchal, Sherrie L Perkins, Hassan M. Yaish, Robert D. Christensen

Br J Haematol. 2016 Sep;174(5):806-14.

➔ Improves precise **diagnosis**, **management** of patients and **counselling** of the patient and their family.

Molecular analysis: Gene panel

- **« In house » panel of 4427 genes (mendeliome)**
  a. Ataxia (524 genes)
  b. Congenital malformation syndromes (853 genes)
  c. Early onset epileptic encephalopathy (836 genes)

  d. Hereditary Hemolytic Anaemias due to unknown or doubtful origin (56 genes)

  e. Hereditary spastic paraplegia (160 genes)
  f. Neurodevelopmental disorders (1376 genes)
  g. Neuromuscular disorders (535 genes)
  h. Dermatogenetic panel, severe, rare and hereditary genodermatoses (374 genes)

LITTERATURE & GUIDELINES

Must-read

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Thank you !

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