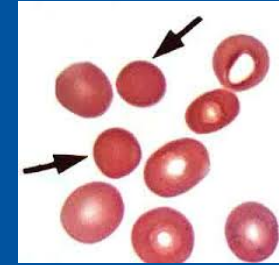




UMC Utrecht



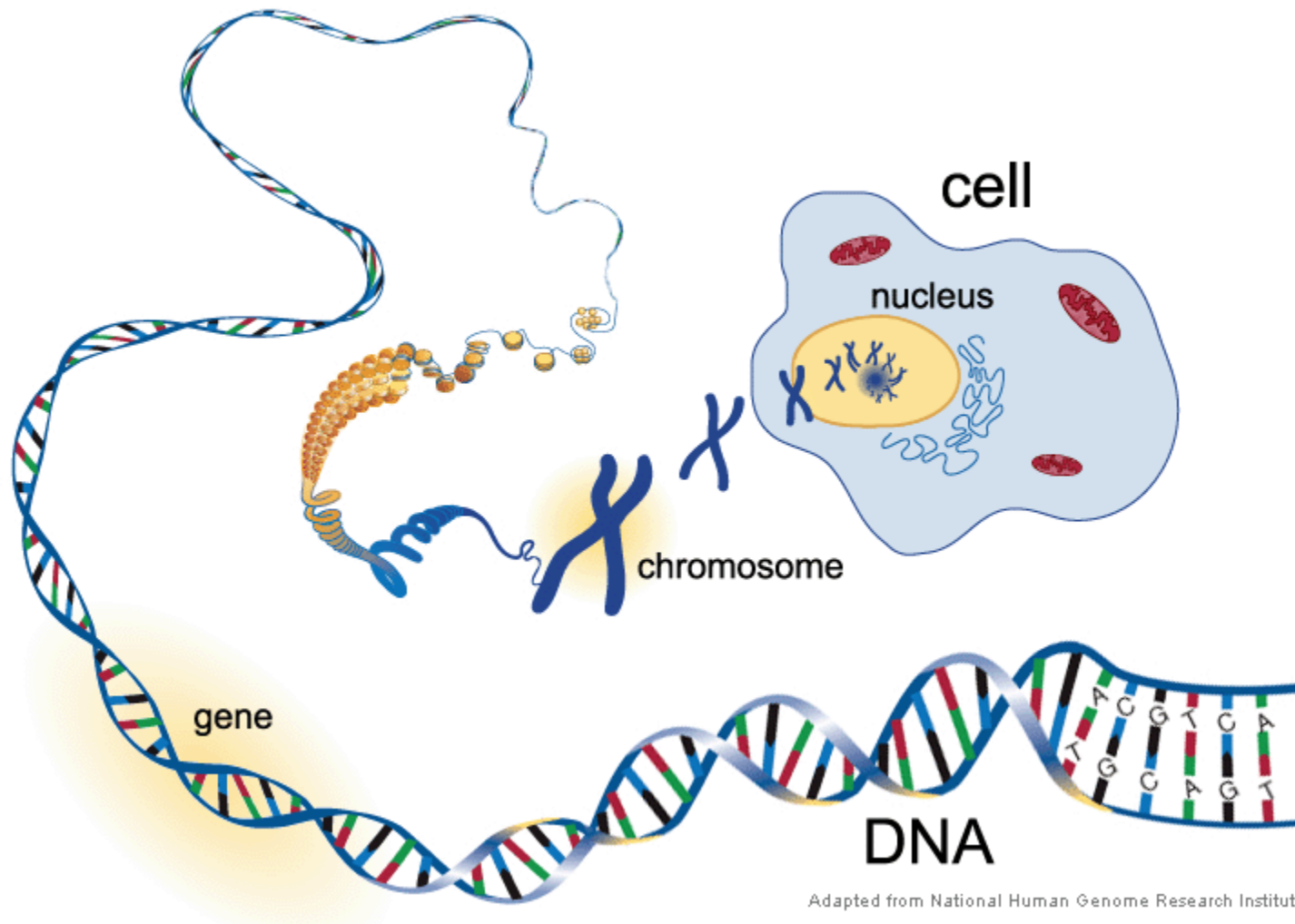
# Is DNA onderzoek belangrijk bij sferocytose?



Marije Bartels, kinderhematoloog



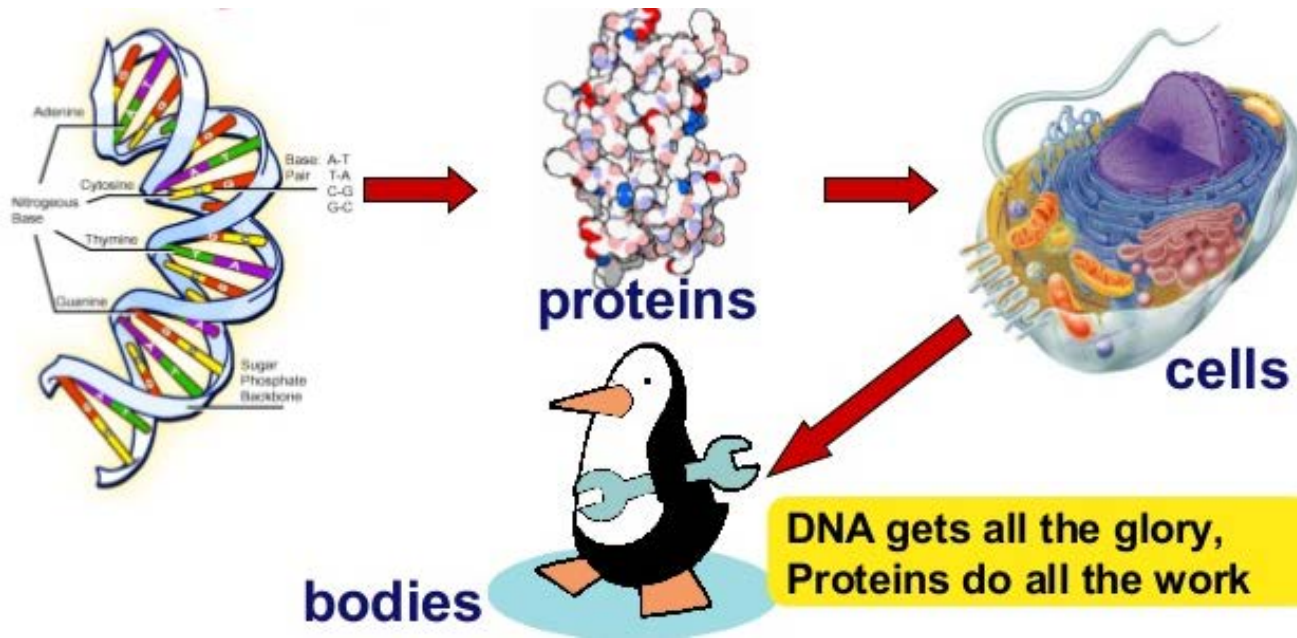
# Wat is DNA onderzoek?



Adapted from National Human Genome Research Institute



# Van DNA naar eiwit

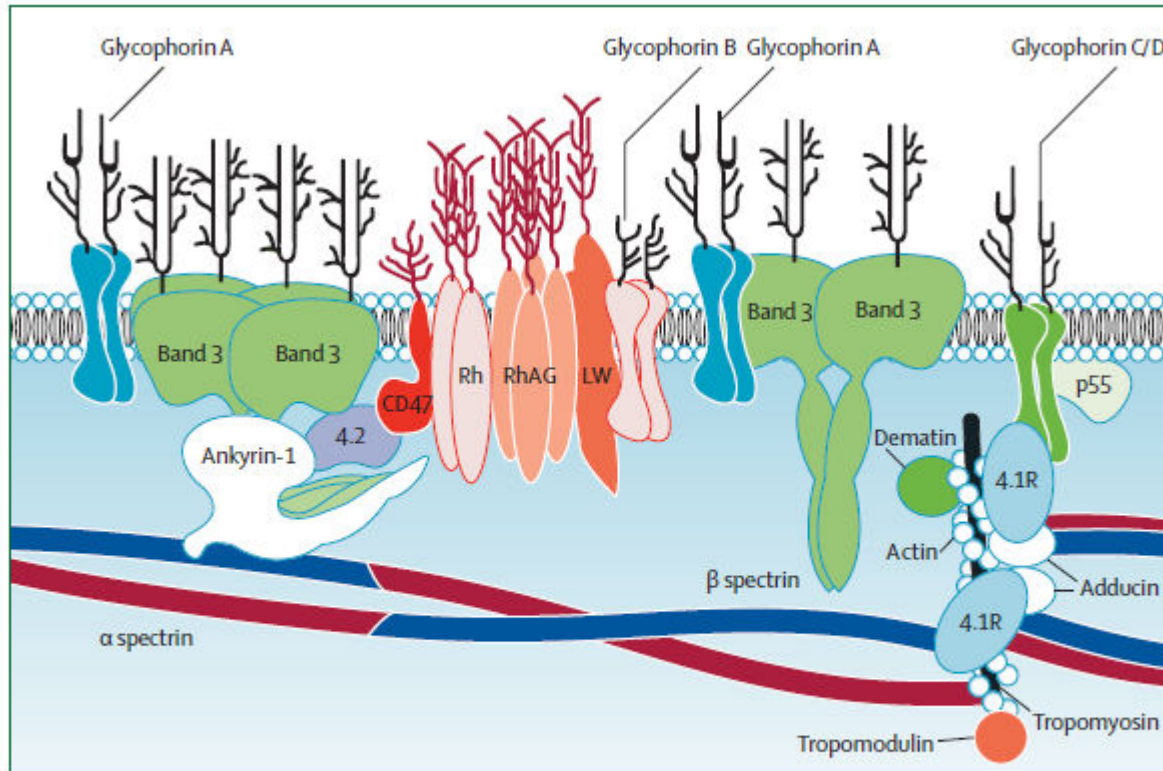


DNA code (gen) → vertaling in eiwitten → functie in de cel

Foutje in DNA (mutatie) → code verkeerd vertaald →  
afwijkend eiwit → gestoorde functie in de cel



# Belangrijke eiwitten bij sferocytose



**Figure 1: A simplified cross-section of the red blood cell (erythrocyte) membrane**

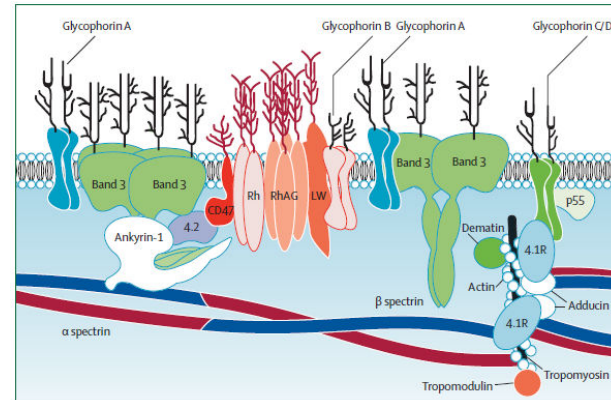
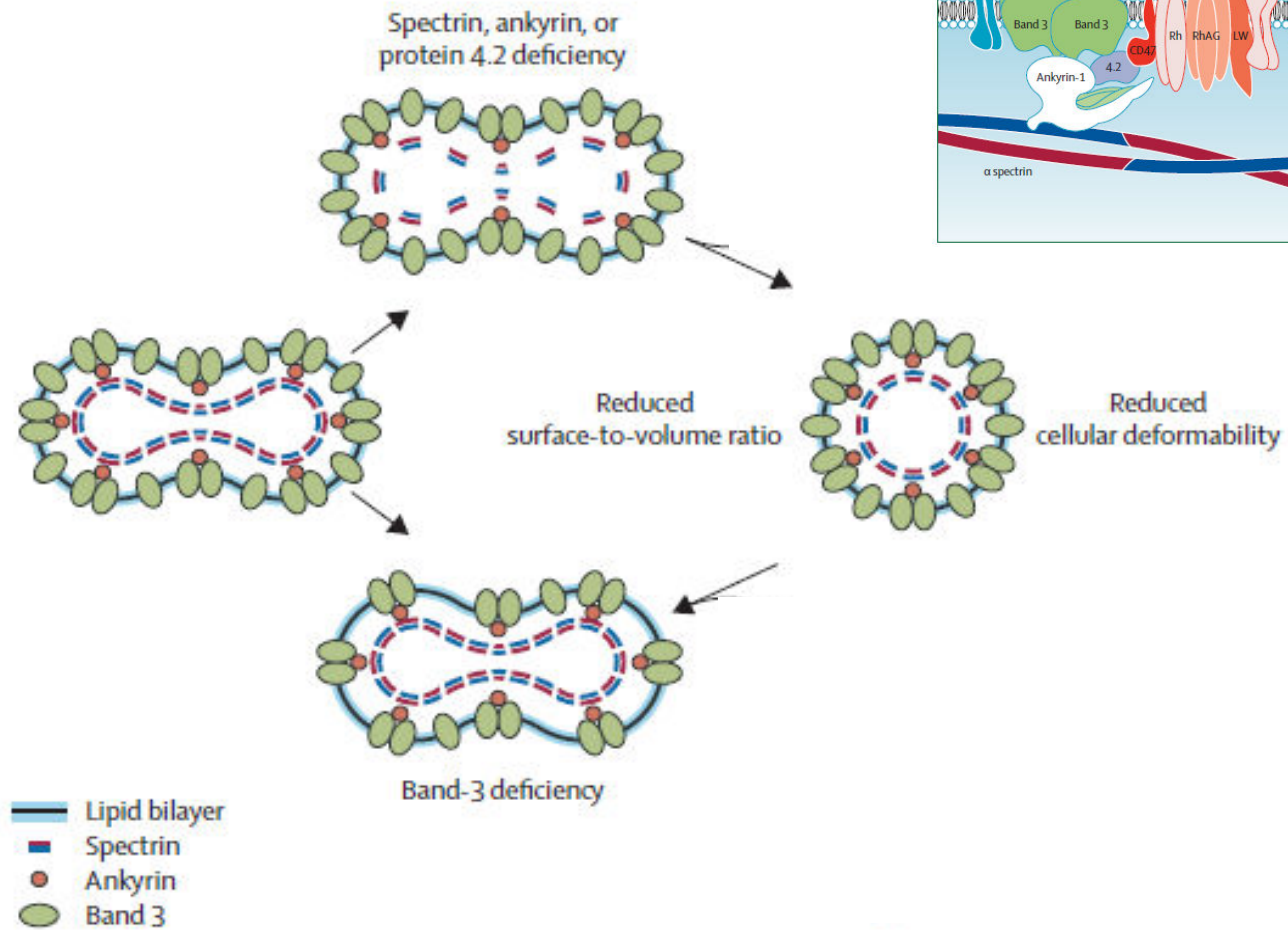
The lipid bilayer forms the equator of the cross-section with its polar heads (small circles) turned outward.

4.1R=protein. 4.2=protein 4.2. Rh=Rhesus polypeptide. RhAG=Rh-associated glycoprotein. LW=Landsteiner-Wiener glycoprotein.

Spectrine (alpha, beta)  
Ankyrine  
Band 3  
Eiwit 4.2

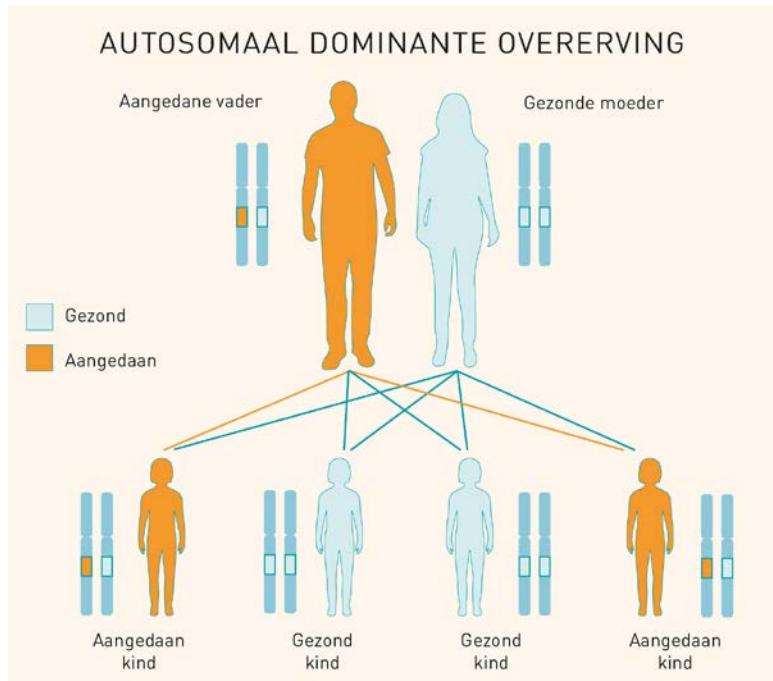


# Belangrijke eiwitten bij sferocytose

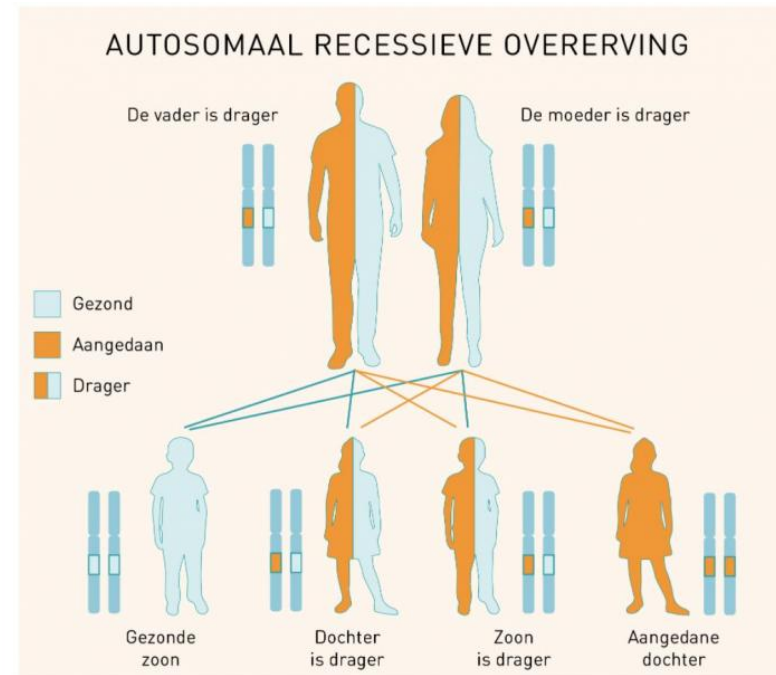




# Erfelijkheid bij sferocytose



50% kans  
 $\beta$ -spectrine  
Band 3  
Ankyrin



25% kans  
 $\alpha$ -spectrine  
Eiwit 4.2  
Ankyrine



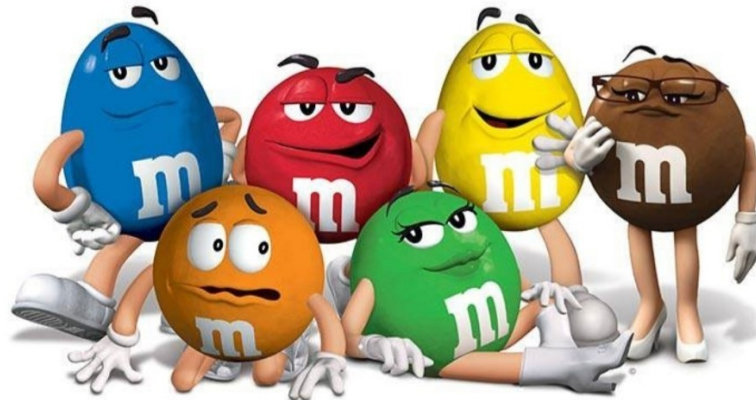
# Waarom doen we DNA onderzoek?

1. Uitleg, advies en preventie
2. Beter karakteriseren van ziektebeeld
3. Relatie met ziekte ernst?
4. Voorspellende waarde?

*Is er een verband tussen het aangedane gen/eiwit en klinische verschijnselen?*

## Genotype-fenotype relatie

Ernst anemie?  
Kans op splenectomie?  
Risico galstenen?  
Etc....



## **The Complexity of Genotype-Phenotype Correlations in Hereditary Spherocytosis: A Cohort of 95 Patients**

### Genotype-Phenotype Correlation in Hereditary Spherocytosis

Annelies van Vuren<sup>1</sup>, Bert van der Zwaag<sup>2</sup>, Rick Huisjes<sup>3</sup>, Nathalie Lak<sup>4</sup>, Marc Bierings<sup>5</sup>, Egbert Gerritsen<sup>6</sup>, Eduard van Beers<sup>7</sup>, Marije Bartels<sup>8</sup>, Richard van Wijk<sup>9</sup>

95 patienten met diagnose sferocytose

Bloedonderzoek

Genetisch onderzoek

Functioneel onderzoek

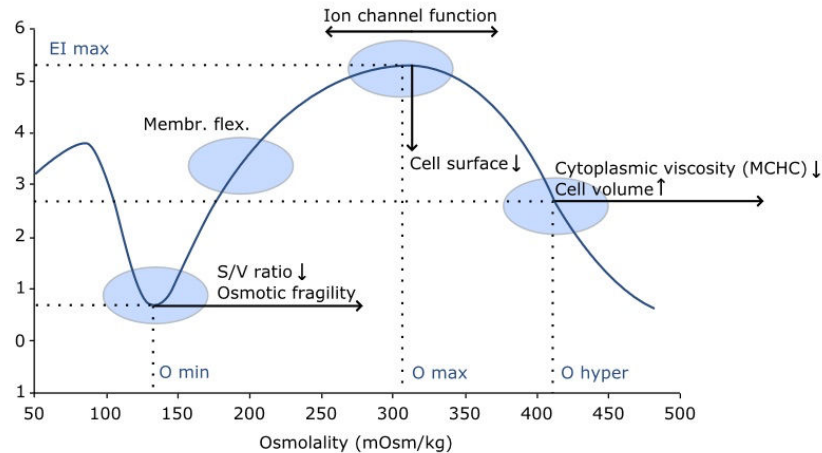


Laser Optical Rotational Red Cell Analyzer

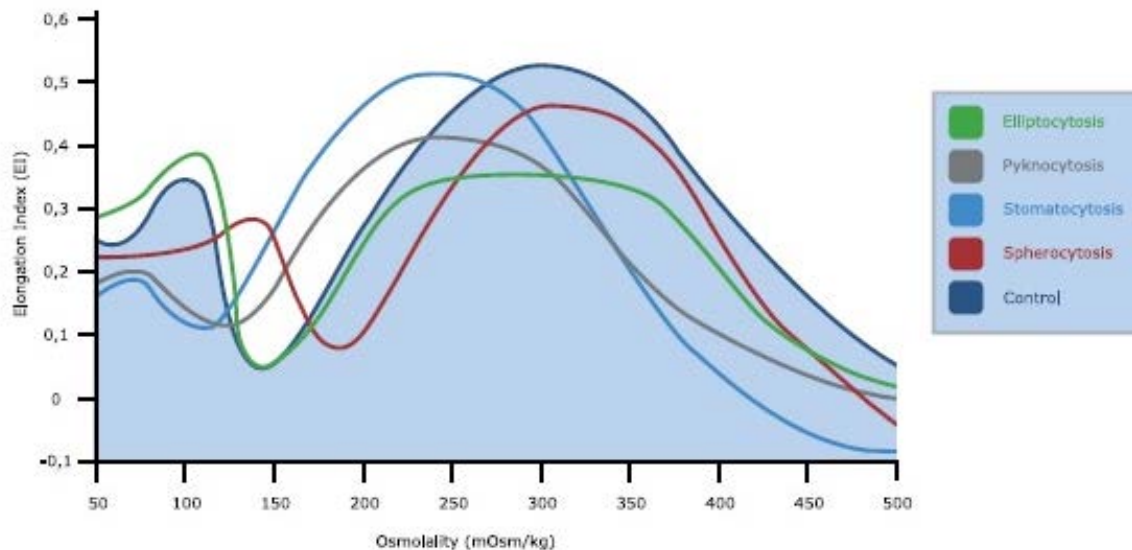




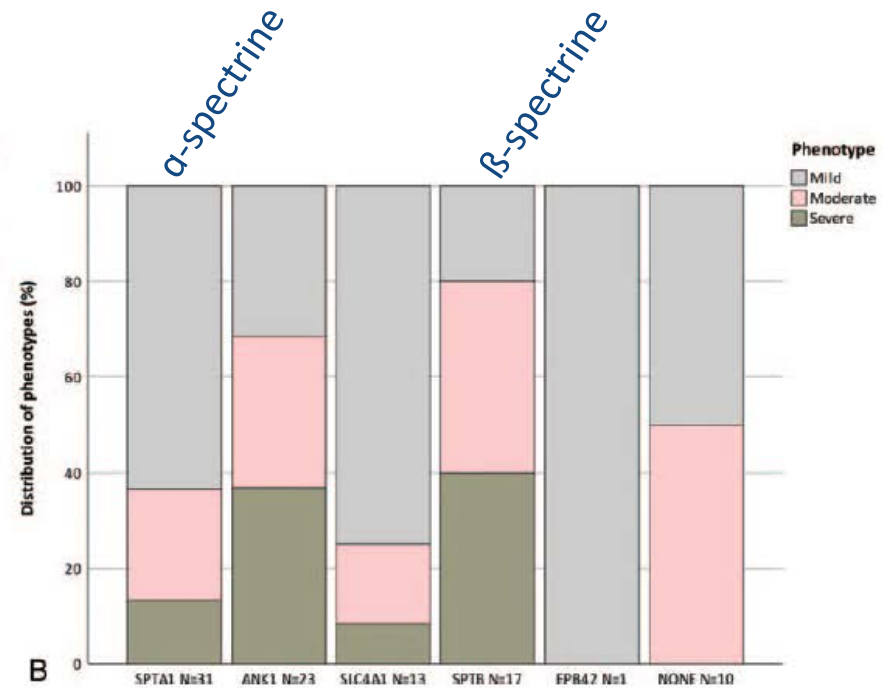
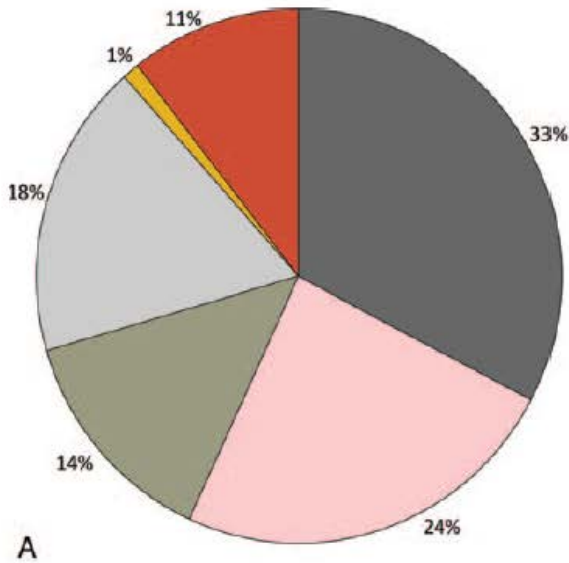
# De verandering in de functie bij sferocytose (en andere aandoeningen)



Typical osmoscan curves



# Onderzoek UMCU



| Table S1. Classification HS based on disease severity |               |               |                                |
|-------------------------------------------------------|---------------|---------------|--------------------------------|
| Classification                                        | Mild          | Moderate      | Severe                         |
| Hemoglobin (g/dL)                                     | 11-15         | 8-12          | <8                             |
| Reticulocyte count (%)                                | 3.1-6         | >6            | >10                            |
| Bilirubin (μmol/L)                                    | 17-34         | >34           | >51                            |
| Splenectomy                                           | Not indicated | Not indicated | Indicated after age of 6 years |

Hb 11 g/dL = 6.8 mmol/L

Hb 8 g/dL = 5.0 mmol/L

**Table S1** Classification HS based on disease severity. The original classification was developed by Eber et al.<sup>27</sup>



## The Complexity of Genotype-Phenotype Correlations in Hereditary Spherocytosis: A Cohort of 95 Patients

### Genotype-Phenotype Correlation in Hereditary Spherocytosis

Annelies van Vuren<sup>1</sup>, Bert van der Zwaag<sup>2</sup>, Rick Huisjes<sup>3</sup>, Nathalie Lak<sup>4</sup>, Marc Bierings<sup>5</sup>, Egbert Gerritsen<sup>6</sup>, Eduard van Beers<sup>7</sup>, Marije Bartels<sup>8</sup>, Richard van Wijk<sup>9</sup>

**Table 3**

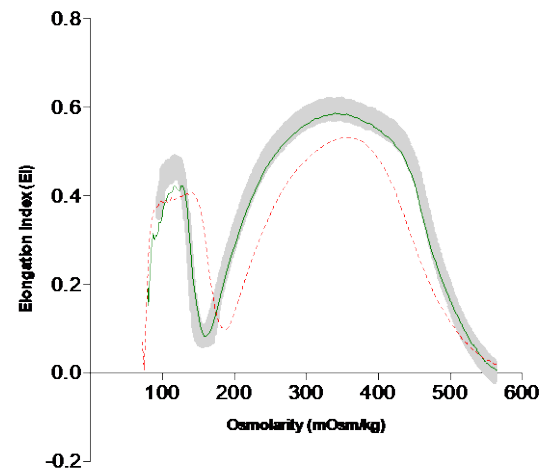
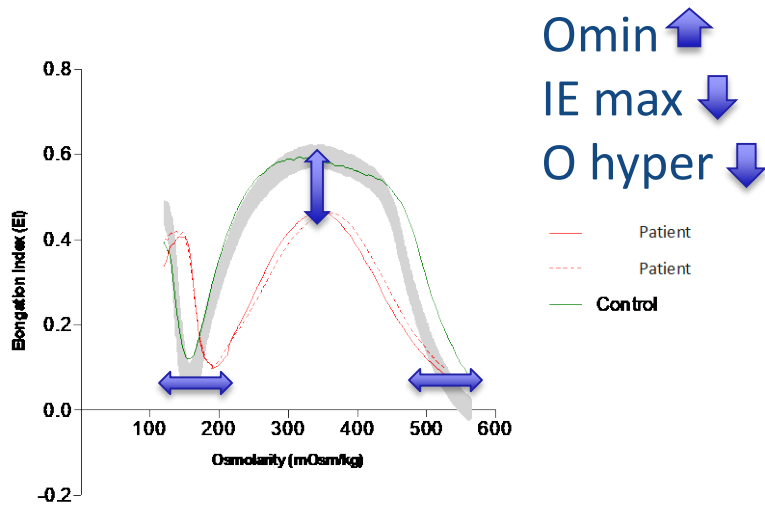
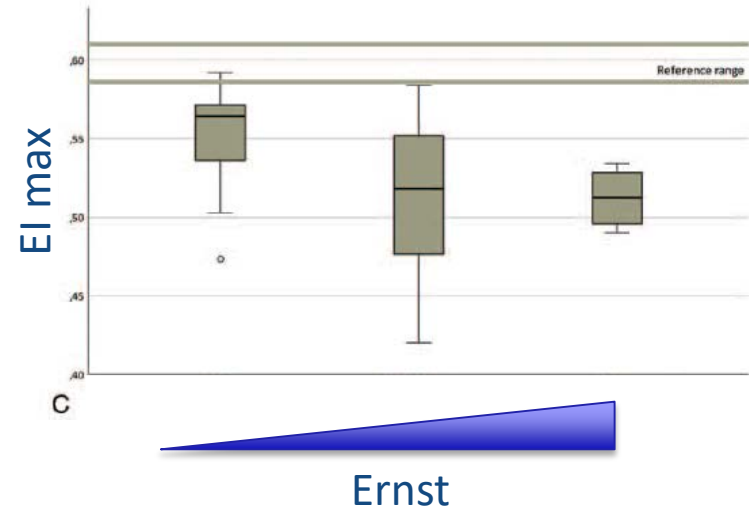
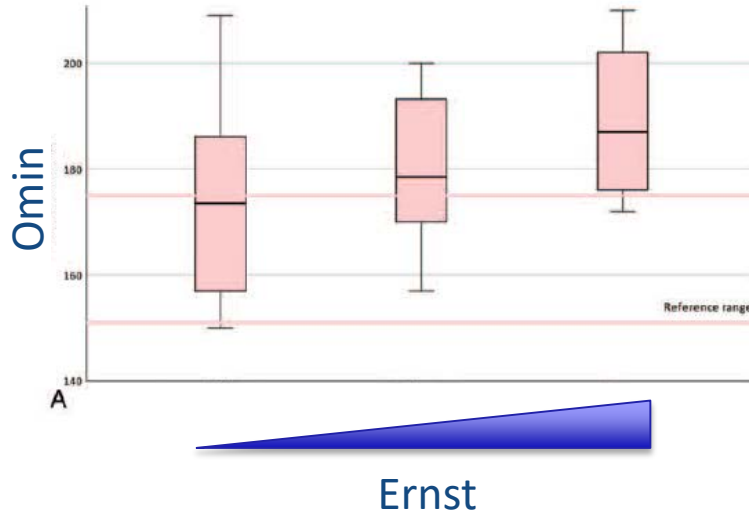
#### Erythropoietic and Hemolytic Parameters per Gene Group.

|                                                    | SPTA1<br>N=26 | ANK1<br>N=15 | SPTB<br>N=9 | SLC4A1<br>N=11 | EPB42<br>N=1 |
|----------------------------------------------------|---------------|--------------|-------------|----------------|--------------|
| Gender (% male)                                    | 46            | 40           | 56          | 64             | 0            |
| <i>Laboratory parameters</i>                       |               |              |             |                |              |
| [reference range]                                  |               |              |             |                |              |
| Hb (g/dL)                                          | 11.7 (2.3)    | 10.8 (2.2)   | 10.5 (2.7)  | 13.2 (1.9)     | 14.5 (-)     |
| [11.9–17.2]                                        |               |              |             |                |              |
| Reticulocyte count. absolute (x10 <sup>9</sup> /L) | 298 (185)     | 506 (203)    | 580 (214)   | 259 (84)       | 210 (-)      |
| [25–120]                                           |               |              |             |                |              |
| LD (U/L)                                           | 274 (119)     | 291 (52)     | 495 (497)   | 214 (34)       | 247 (-)      |
| [0–250]                                            |               |              |             |                |              |
| Bilirubin (μmol/L)                                 | 52 (53)       | 50 (17)      | 47 (20)     | 39 (14)        | 11 (-)       |
| [3–21]                                             |               |              |             |                |              |
| AST (U/L)                                          | 33 (15)       | 31 (15)      | 27 (5)      | 24 (5)         | NA           |
| [0–35]                                             |               |              |             |                |              |
| Ferritin (μg/L)                                    | 415 (758)     | 381 (303)    | 306 (199)   | 342 (411)      | 31 (-)       |
| [20–250]                                           |               |              |             |                |              |
| <i>HS diagnostics</i>                              |               |              |             |                |              |
| EMA (%)                                            | 87 (13)       | 75 (9)       | 72 (8)      | 74 (8)         | 88 (-)       |

Mean values are presented (±standard deviation). Mean values in hemoglobin ( $p < 0.05$ ), absolute reticulocyte count ( $p < 0.01$ ) and EMA ( $p < 0.05$ ) differed significantly between the categories displayed in bright and light orange. EMA=eosin-5-maleimide binding test; NA=no available data.



# Functietesten voorspellen klinische ernst....redelijk



# Maar er zijn ook andere....nog onbekende factoren....

## Family I

■ *SPTB*: c.3449G>A p.(Trp1150\*) (5-P)  
■ α-LELY

Post-splenectomy:  
no anemia,  
persistent reticulocytosis.  
Ektacytometry: classical HS.

No anemia,  
no reticulocytosis.  
Ektacytometry: normal.

I  
9 y.  
Pre-splenectomy: severe  
anemia and reticulocytosis.  
Transfusion dependent.  
Splenectomy age 6 y.  
Post-splenectomy: no anemia.  
Ektacytometry: classical HS.

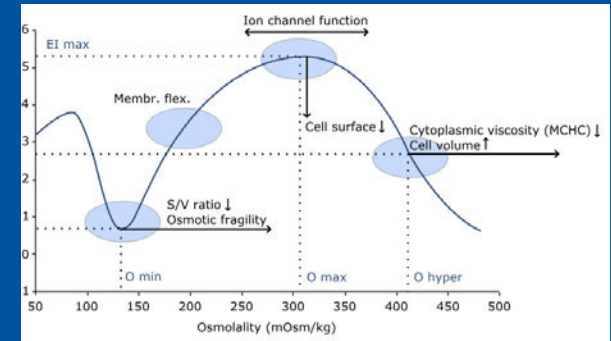
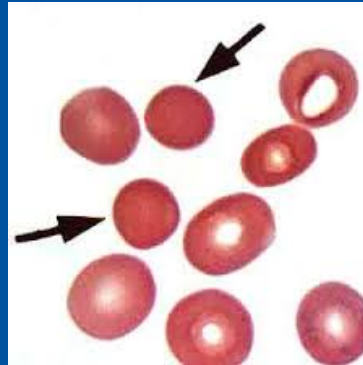
II  
7 y.  
Mild anemia. No red cell  
transfusions.  
Ektacytometry: HS, right-  
shifted  $O_{\text{hyper}}$  pattern  
compared to other family  
members.

III  
2 y.  
Severe anemia,  
reticulocytosis. Transfusion  
dependent.  
Ektacytometry: classical HS.

B



# Is DNA onderzoek belangrijk bij sferocytose?

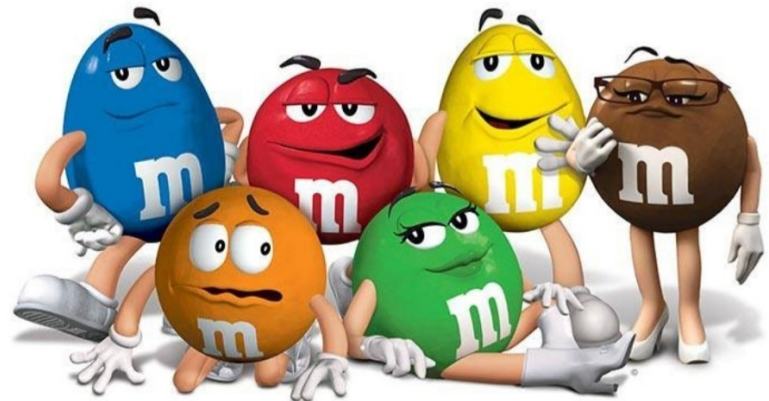




# Onderzoek doen is belangrijk, maar.....



RBC onderzoeksgroep (Richard van Wijk)



- TAPIR studie
- Effecten van splenectomie op RBC “gedrag”
- “Metabolomics” in zeldzame anemie
- Mechanismen van ijzerstapeling bij zeldzame anemie

