EUROPEAN HEMATOLOGY ASSOCIATION

NHS Oxford University Hospitals NHS Foundation Trust

Clinical Usefulness of NGS in diagnosis of Hereditary Haemolytic Anaemias

Dr Noémi Roy

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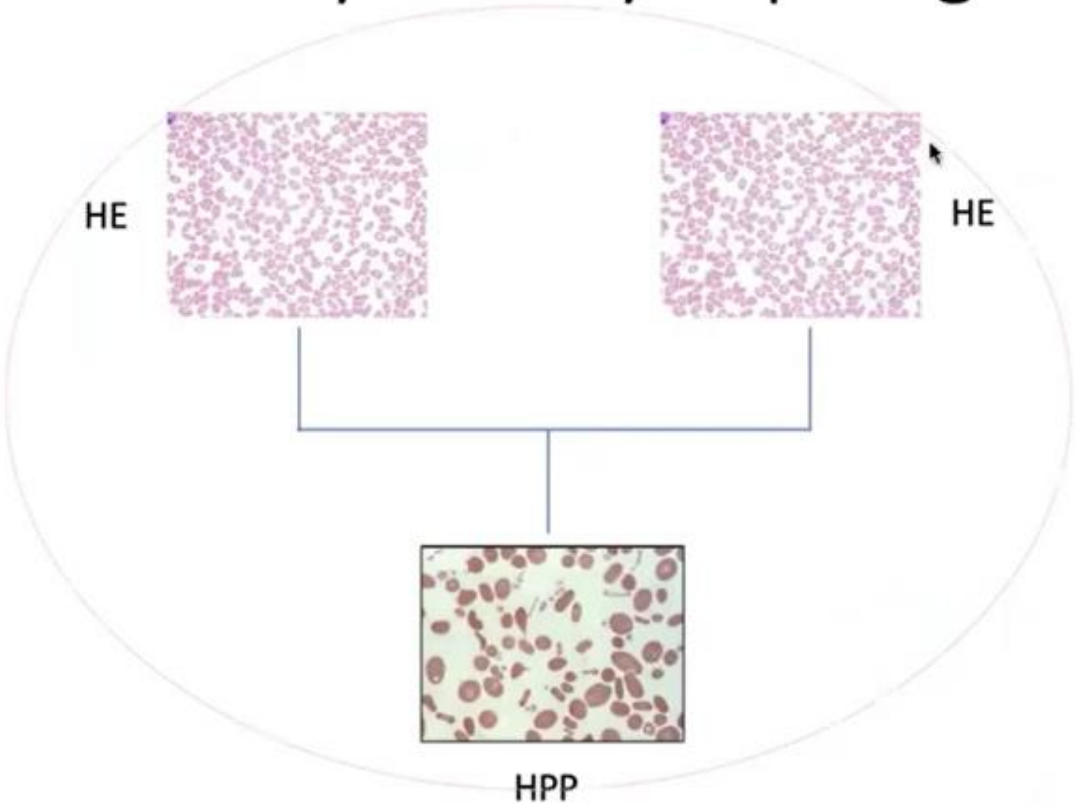
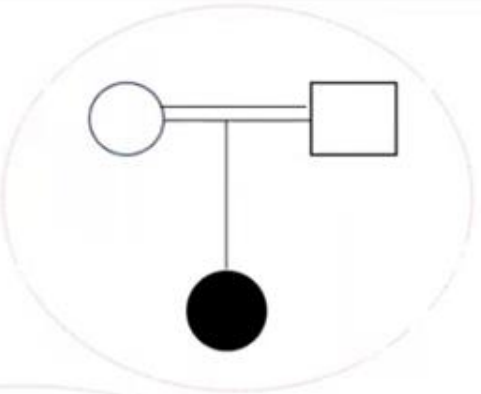
Oxford University Hospitals NHS Foundation Trust

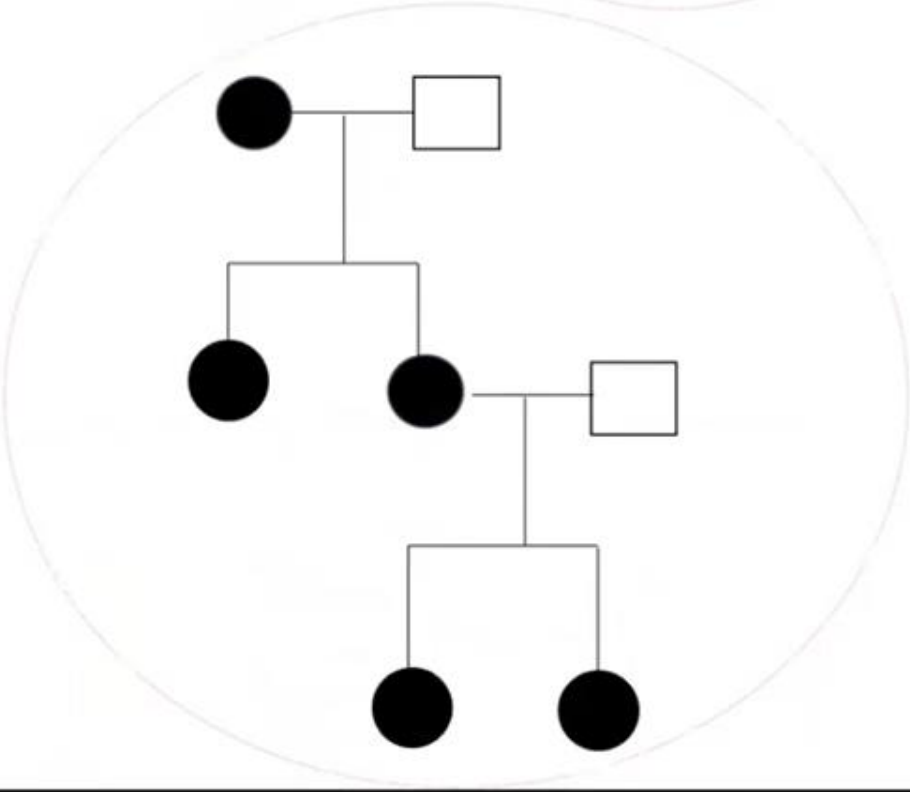
Weatherall Institute of Molecular Medicine

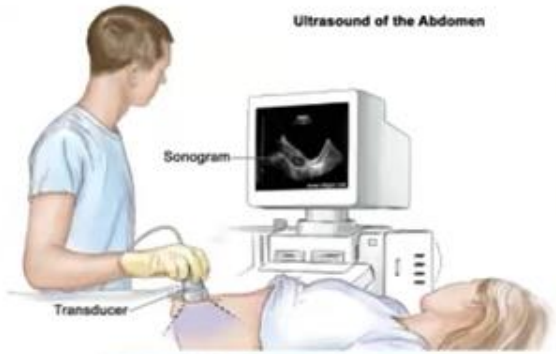
University of Oxford

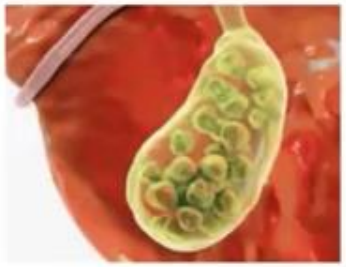
Family history & pedigrees

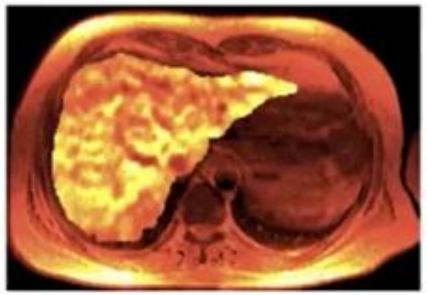






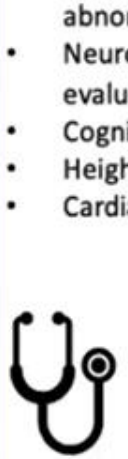






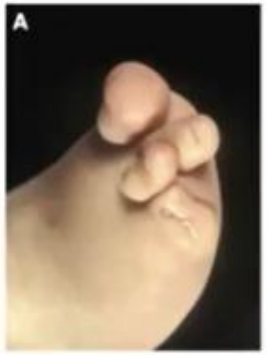
EXAMINATION

- Jaundice
- Hepatosplenomegaly
- Limb or extremities abnormalities
- Neuromuscular evaluation
- Cognitive level
- Height
- Cardiac anomalies

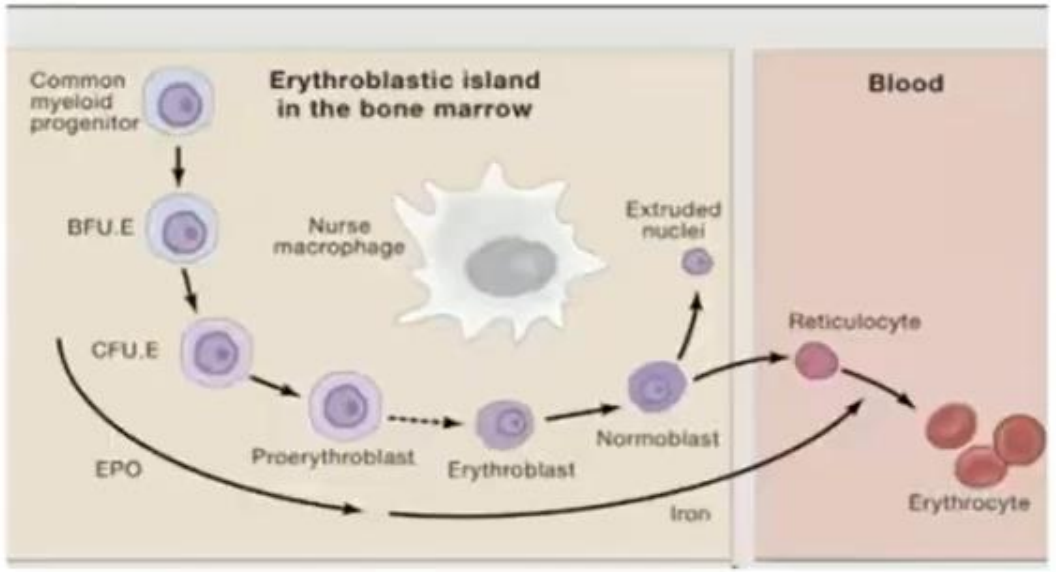


Normal spleen

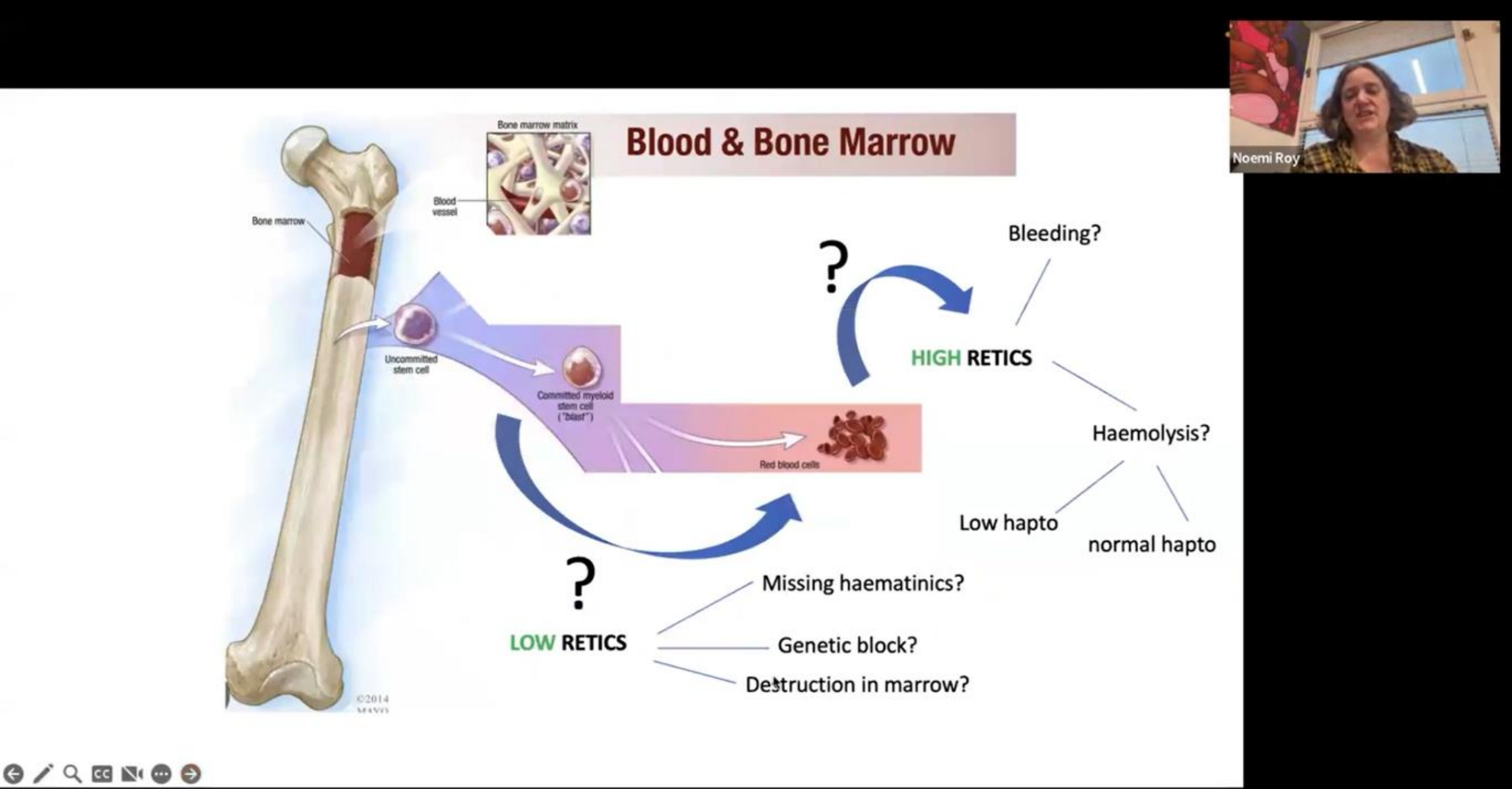
Splenomegaly



Normal erythropoiesis



Cell. 2017 Jan 26; 168(3): 344–361. doi: 10.1016/j.cell.2016.12.034


Blood & Bone Marrow
Bone marrow matrix
Blood vessel
Bone marrow
Uncommitted stem cell
Committed myeloid stem cell ("blast")
Red blood cells
?
Bleeding?
HIGH RETICS
Haemolysis?
Low hapto
normal hapto
?
Missing haematinics?
LOW RETICS
Genetic block?
Destruction in marrow?
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Hb

MCV

MCH/MCHC

DAT

retics

LDH

haptoglobins

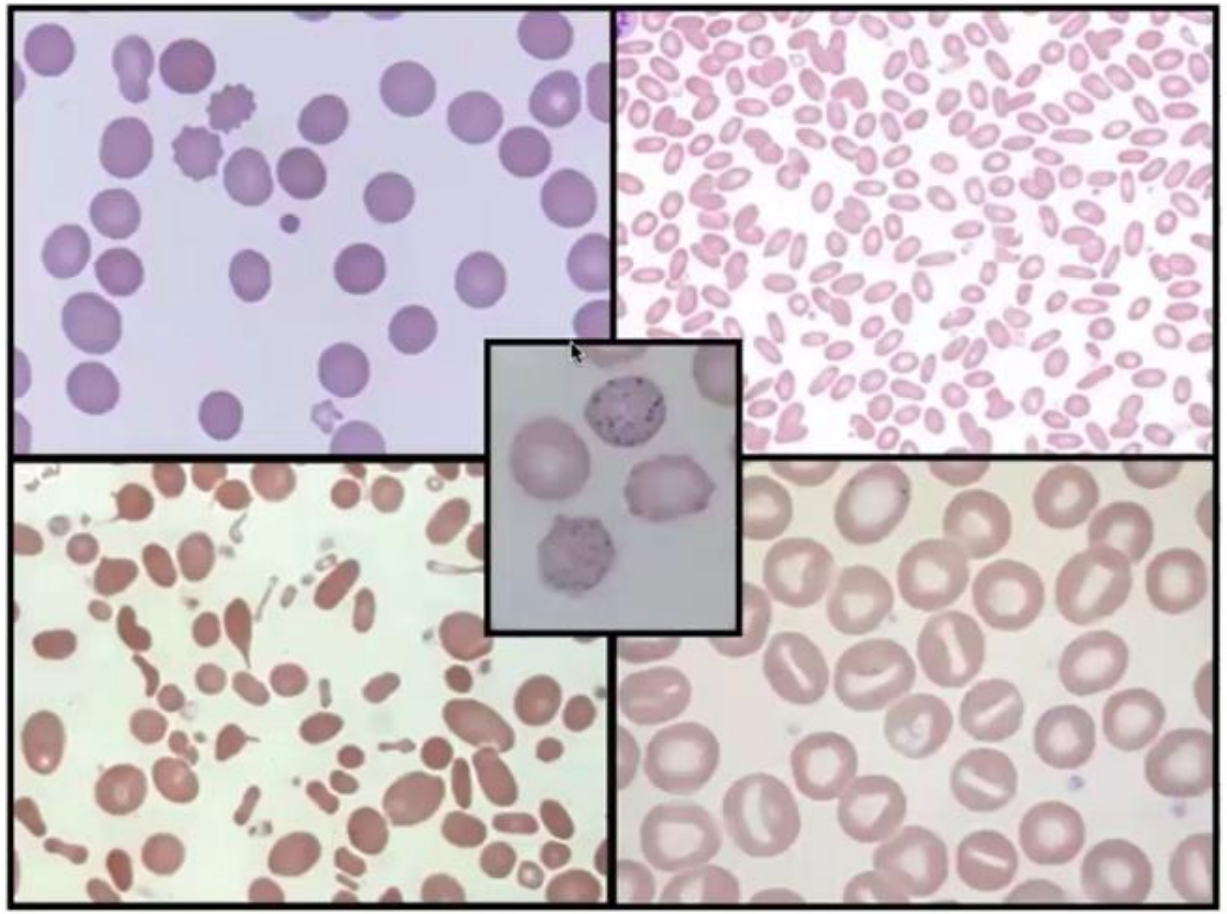
BR

BLOOD TESTS

- FBC
- Retics
- U&Es
- LFTs
- LDH
- Blood film
- HPLC

HBA1c vs fructosamine

Blood films!

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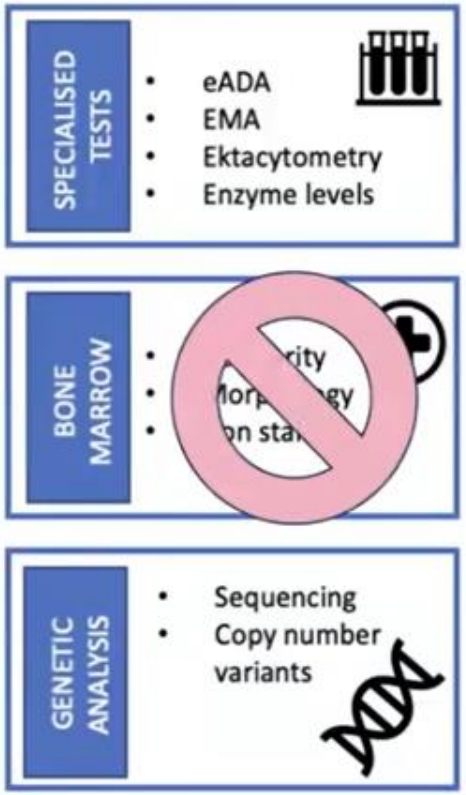
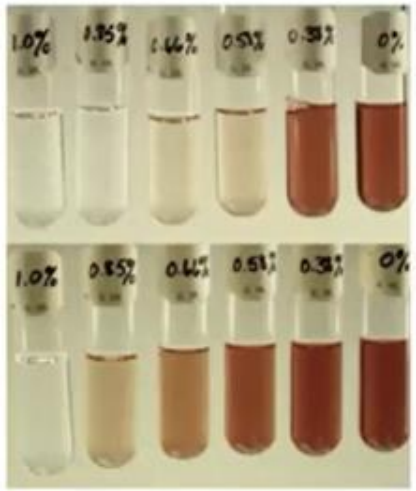
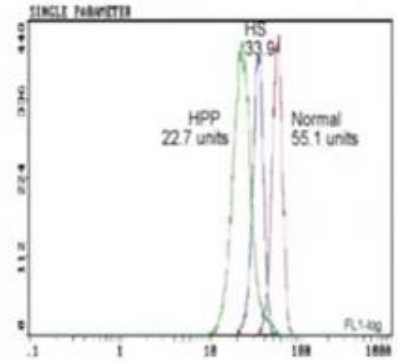
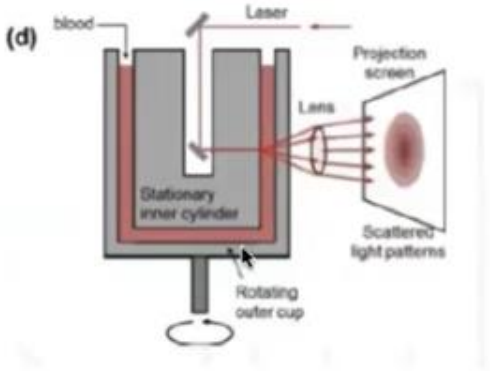

SPECIALISED TESTS
eADA
EMA
Ektacytometry
Enzyme levels
BONE MARROW
GENETIC ANALYSIS
Sequencing
Copy number variants



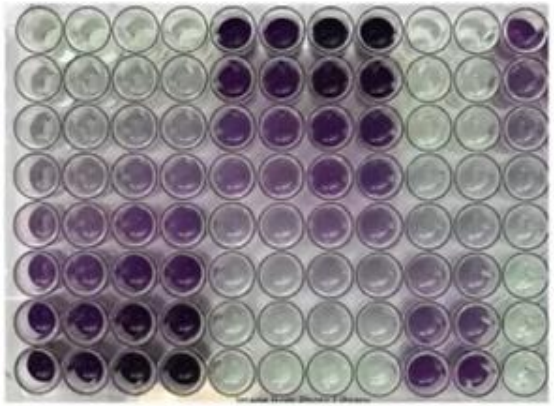
Panel A Graded Fluorescence

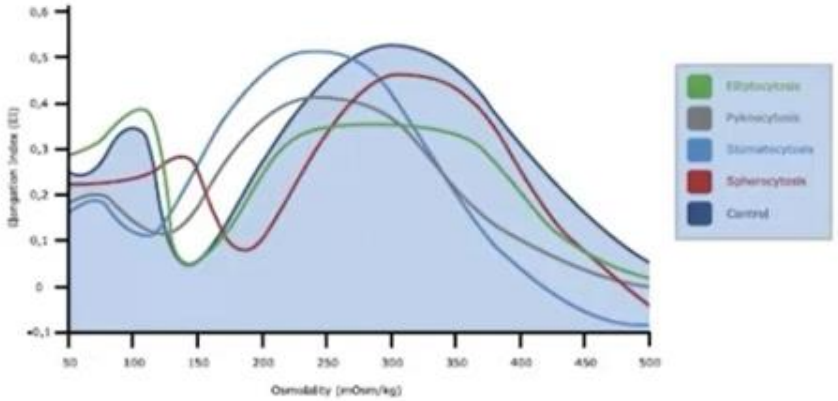




SPECIALISED TESTS

- eADA
- EMA
- Ektacytometry
- Enzyme levels





Quick, less expensive

Need fresh (native) blood, availability & transport

SPECIALISED TESTS

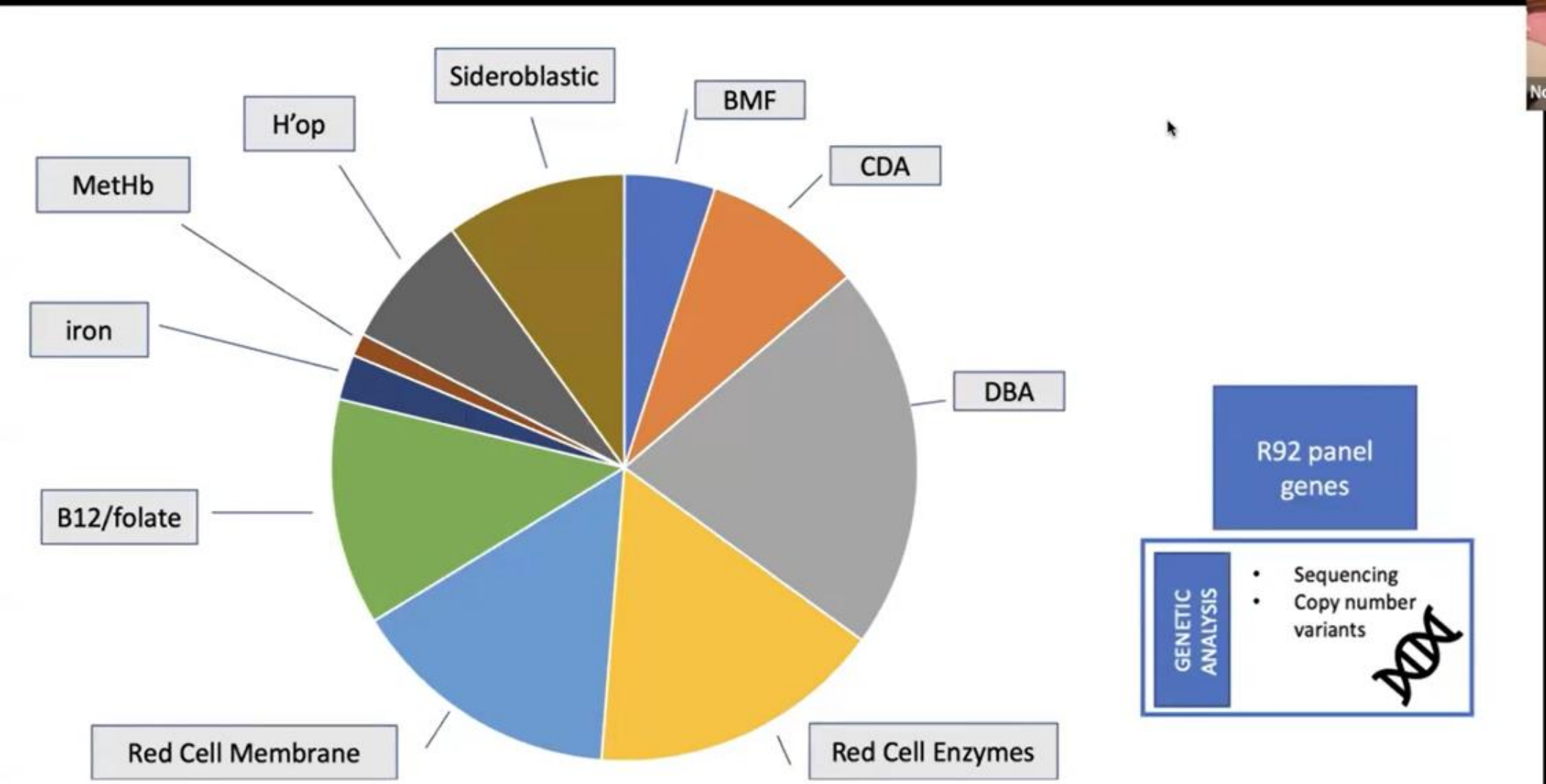
- eADA
- EMA
- Ektacytometry
- Enzyme levels

Can use in transfused patients

Expensive, result takes weeks/months

GENETIC ANALYSIS

- Sequencing
- Copy number variants


Sideroblastic
BMF
H'op
CDA
MetHb
iron
DBA
B12/folate
Red Cell Membrane
Red Cell Enzymes
R92 panel genes
GENETIC ANALYSIS
Sequencing
Copy number variants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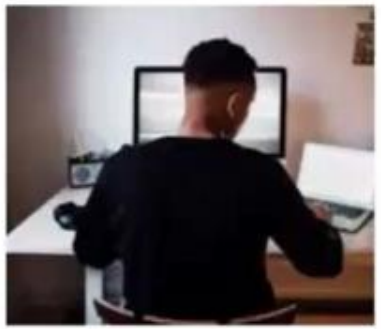

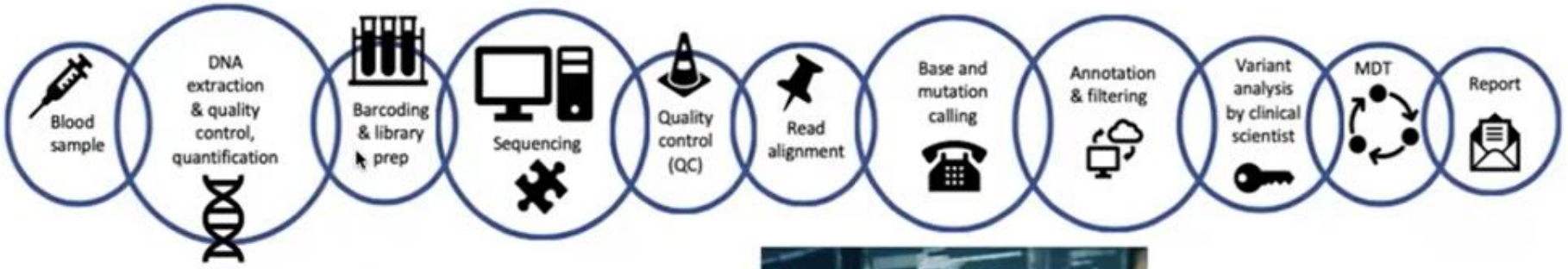
Blood sample
DNA extraction & quality control, quantification
Barcoding & library prep
Sequencing
Quality control (QC)
Read alignment
Base and mutation calling
Annotation & filtering
Variant analysis by clinical scientist
MDT
Report


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Variant classification (ACMG)

Class 5: Pathogenic

Class 4: Likely pathogenic

Class 3: Variant of uncertain significance (VUS)

Class 2: Likely benign

Class 1: Benign

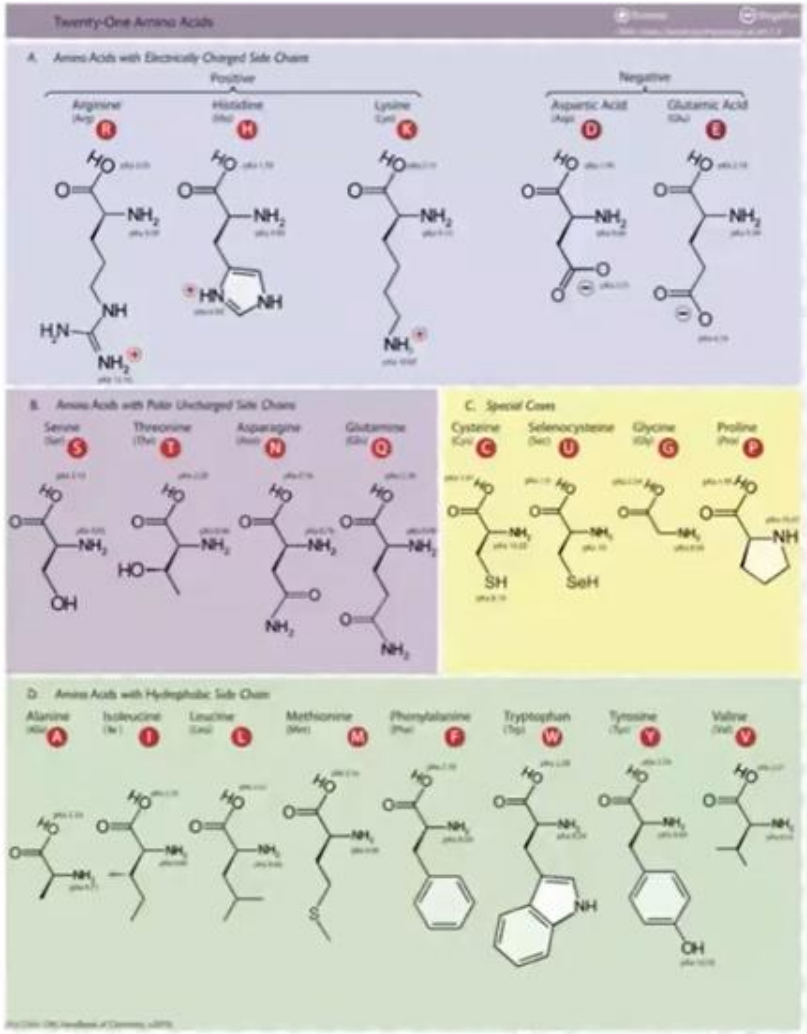
ACMG
American College of Medical Genetics and Genomics

Variant classification (ACMG)

-

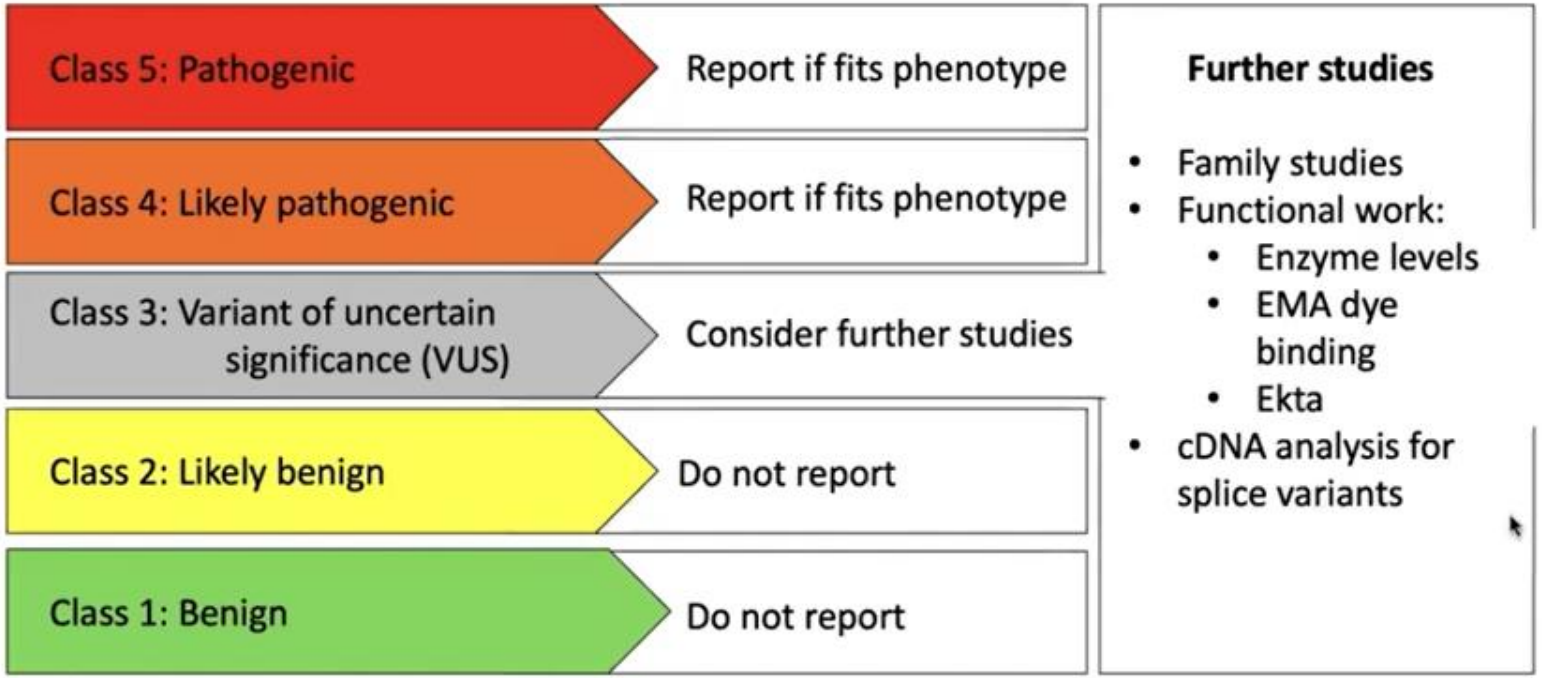
Class 3: Variant of uncertain significance (VUS)

What do predictive software say?



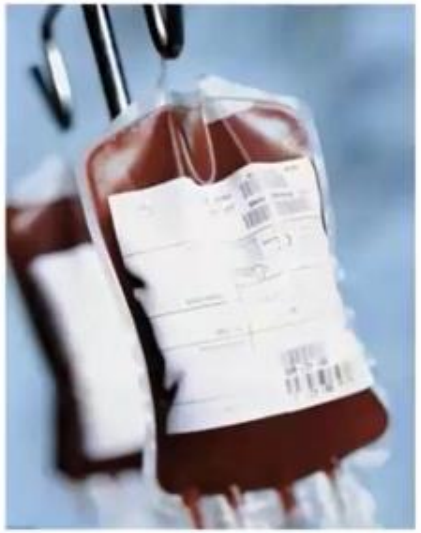
Organism	 CHIMP	 MOUSE	 CHICKEN	 FRUIT FLY
Gene Conservation with Humans (%)	99.5	88	75	60

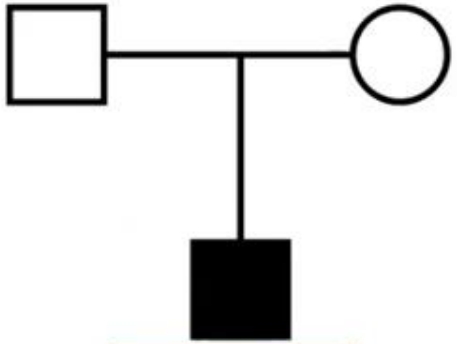
Variant classification (ACMG)



Case 1

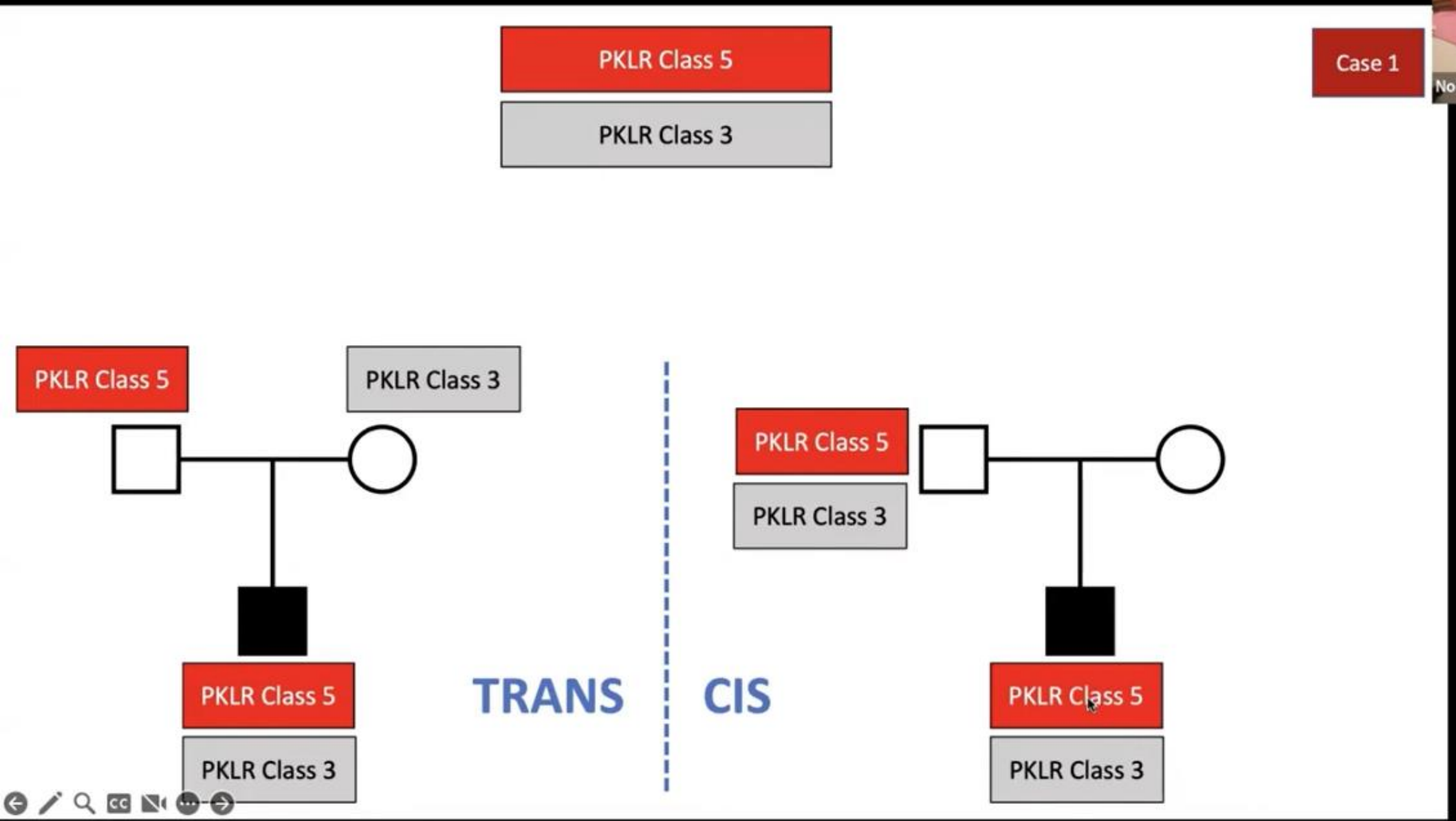
Unexplained transfusion dependent haemolytic anaemia





PKLR Class 5

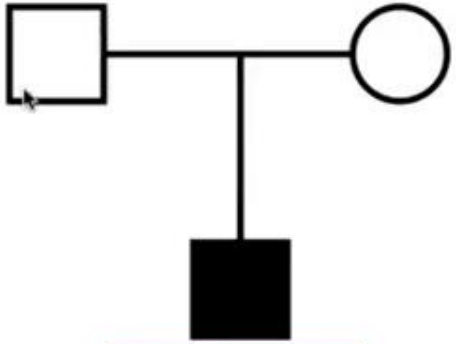
PKLR Class 3


PKLR Class 5
PKLR Class 3
Case 1
Noemi Roy
PKLR Class 5
PKLR Class 3
PKLR Class 5
PKLR Class 3
TRANS
CIS
PKLR Class 5
PKLR Class 3
PKLR Class 5
PKLR Class 3

Case 1

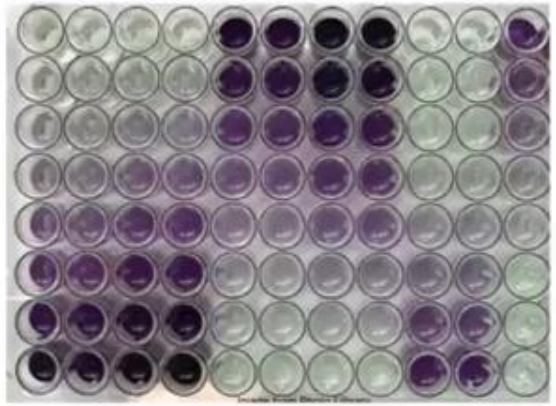
PKLR Class 5

PKLR Class 3

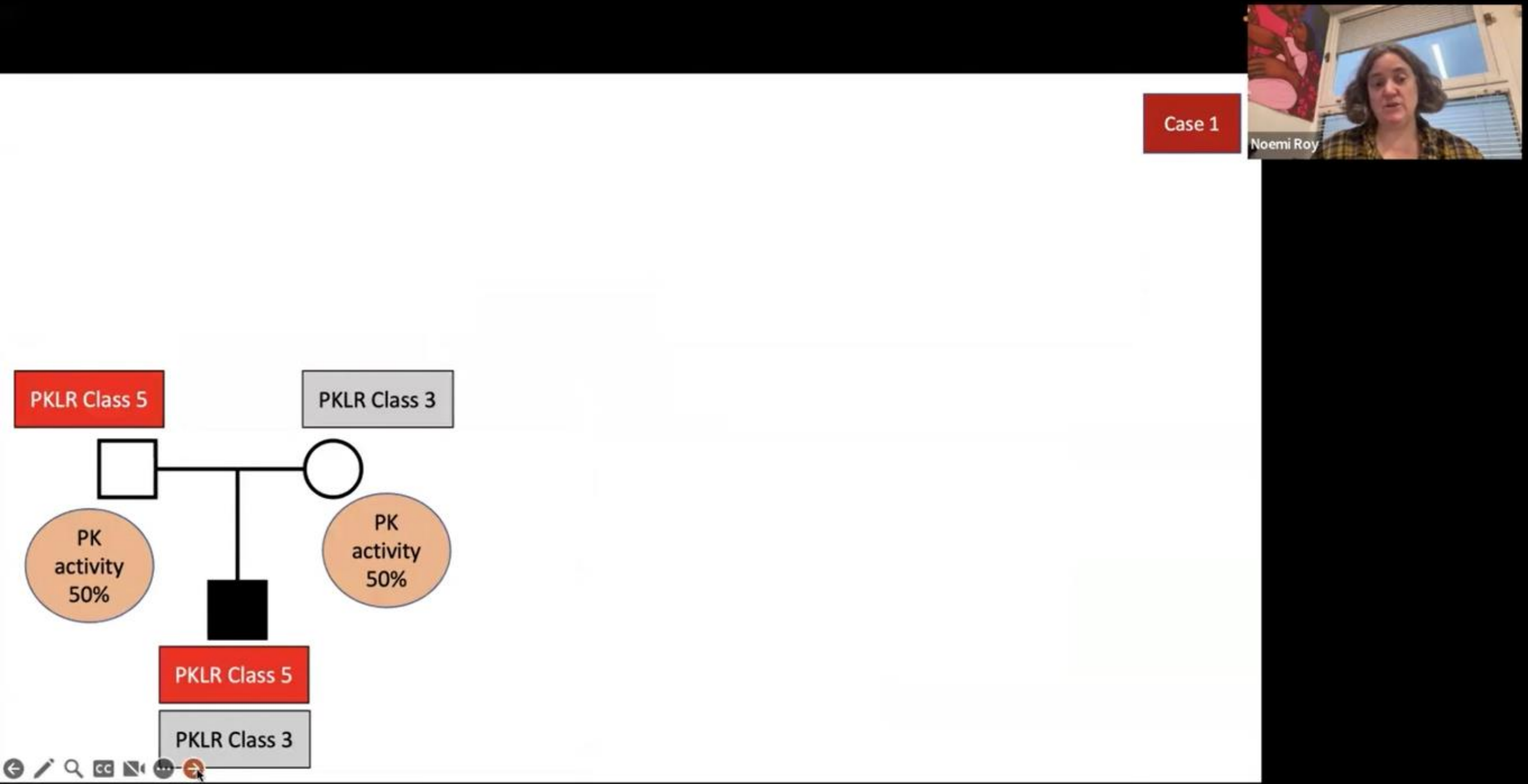


PKLR Class 5

PKLR Class 3



Case 1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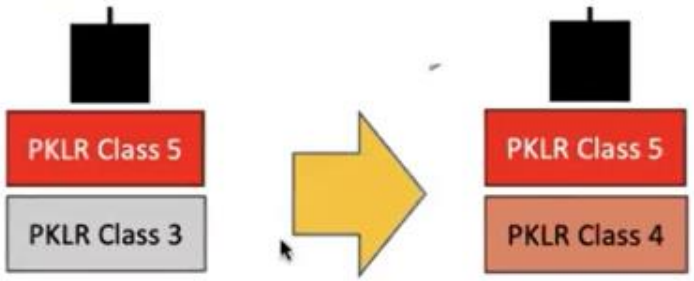
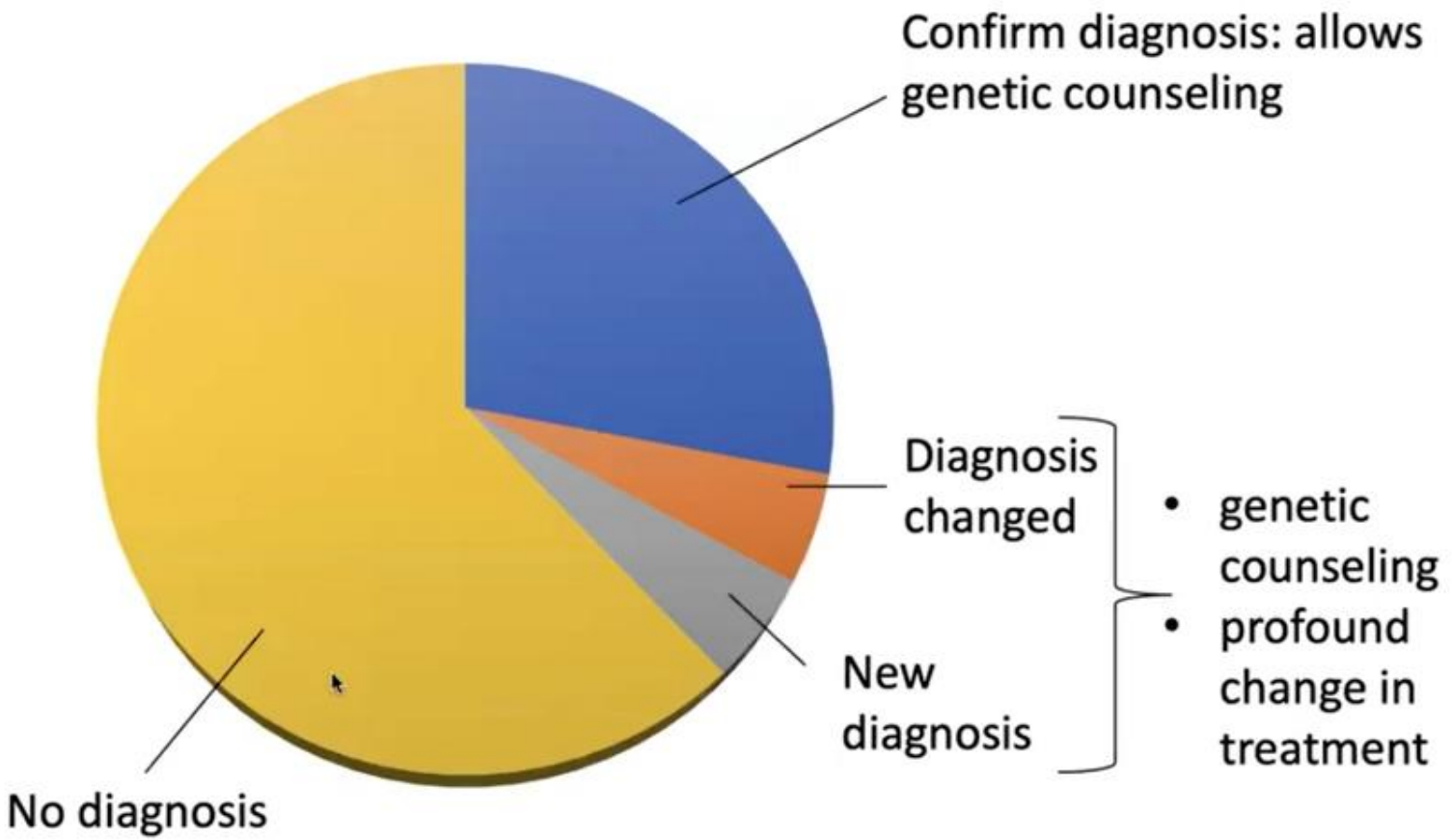
PKLR Class 5
PKLR Class 3
PK activity 50%
PK activity 50%
PKLR Class 5
PKLR Class 3

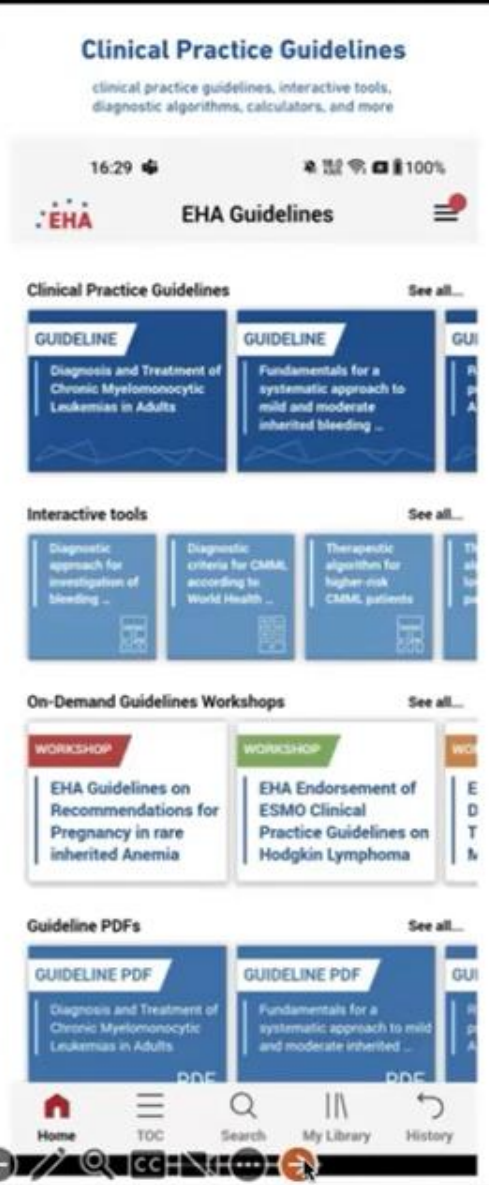
How should variants identified be stored/shared between labs?

- Labs should make a reasonable effort to share variants with other labs analysing the same genes
- Labs should ensure they are using commonly used transcripts which have been shown to be expressed in erythroblasts.



Targeted NGS clinical impact





Good Practice Paper on NGS use in rare inherited anaemia

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DOI: 10.1111/bjh.18191

BJHaem

GUIDELINE

The use of next-generation sequencing in the diagnosis of rare inherited anaemias: A Joint BSH/EHA Good Practice Paper*

Noémi B. A. Roy[1,2] | Lydie Da Costa[3] | Roberta Russo[4,5] | Paola Bianchi[6] | Maria del Mar Mañú-Pereira[7] | Elisa Fermo[6] | Immacolata Andolfo[4,5] | Barnaby Clark[13] | Melanie Proven[8] | Mayka Sanchez[9,10] | Richard van Wijk[11] | Bert van der Zwaag[11] | Mark Layton[12] | David Rees[13] | Achille Iolascon[4,5] | British Society for Haematology/European Hematology Association

Summary

- Should always start with a very thorough history & examination
- Importance of basic tests being done and interpreted first
- Genomics plays a key role, but specialized tests are an important complement
- Accumulating and sharing data on genetic variants and phenotypes will allow us to make better genotype-phenotype correlations in the future → individualized medicine