University of BRISTOL

NHS Blood and Transplant

A serological and molecular study of a novel *XK* inactivating mutation in McLeod syndrome

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BIO-RAD

BBTS 2012, Harrogate, UK

Case history

- 55 year old male patient of Caucasian origin, A rr K-
- No major medical problems, no previous transfusions
- Knee replacement surgery
- Received 1 unit of RBCs due to post-surgical complications
- Presented with an alloantibody to high incidence antigen

Initial serology

Patient's serum:

- Alloantibody to unknown high incidence antigen
- Compatible only with McLeod cells
- Reacted weakly with Ko (Kell null) cells
- Incompatible with all other tested cells
- Additionally, anti-S confirmed

Patient's cells:

- Reduced expression of all Kell antigens
- Compatible with anti-KL

McLeod phenotype?

McLeod phenotype

- Absence of Kx antigen caused by the lack of Kx red cell surface protein
- Reduced expression of all high incidence Kell system antigens
- Absence of Km (KEL20) Kell system antigen
- Acanthocytosis in the peripheral blood

Usually associated with McLeod syndrome (MLS)

McLeod syndrome

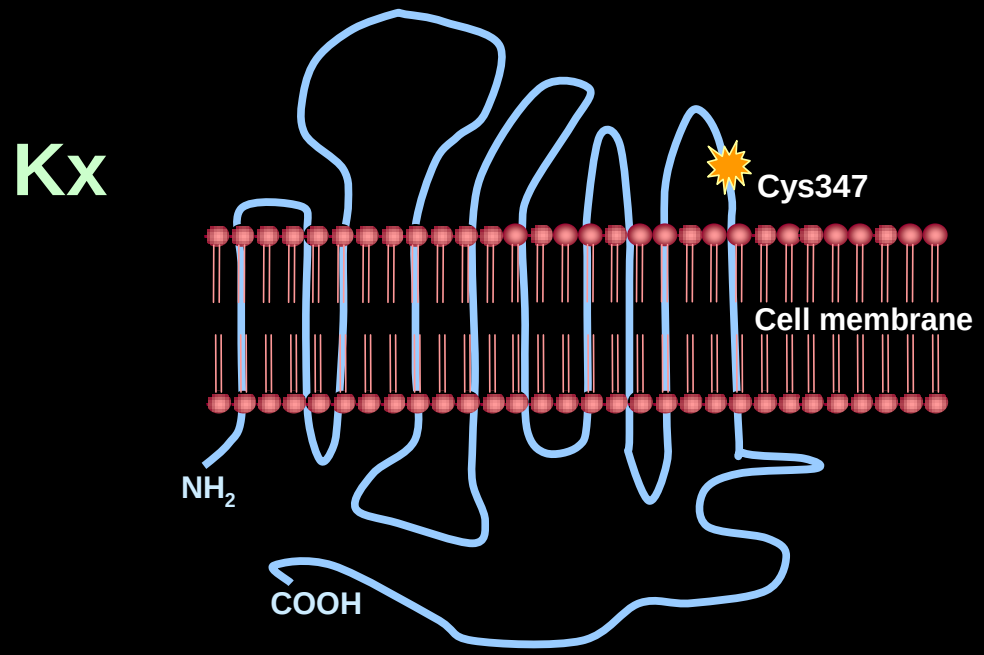
Rare multisystem disorder

Only ~ 150 cases reported worldwide

- Erythrocyte acanthocytosis
- Compensated haemolytic anaemia
- Elevated serum creatine phosphokinase
- Neurological and neuromuscular disorders
- Psychiatric problems
- In some cases can lead to premature death

Kx antigen

- One antigen of Kx blood group system
- Carried on Kx, RBC membrane protein of 37 KDa, 444 aa
- Function not yet known, potentially a membrane transporter

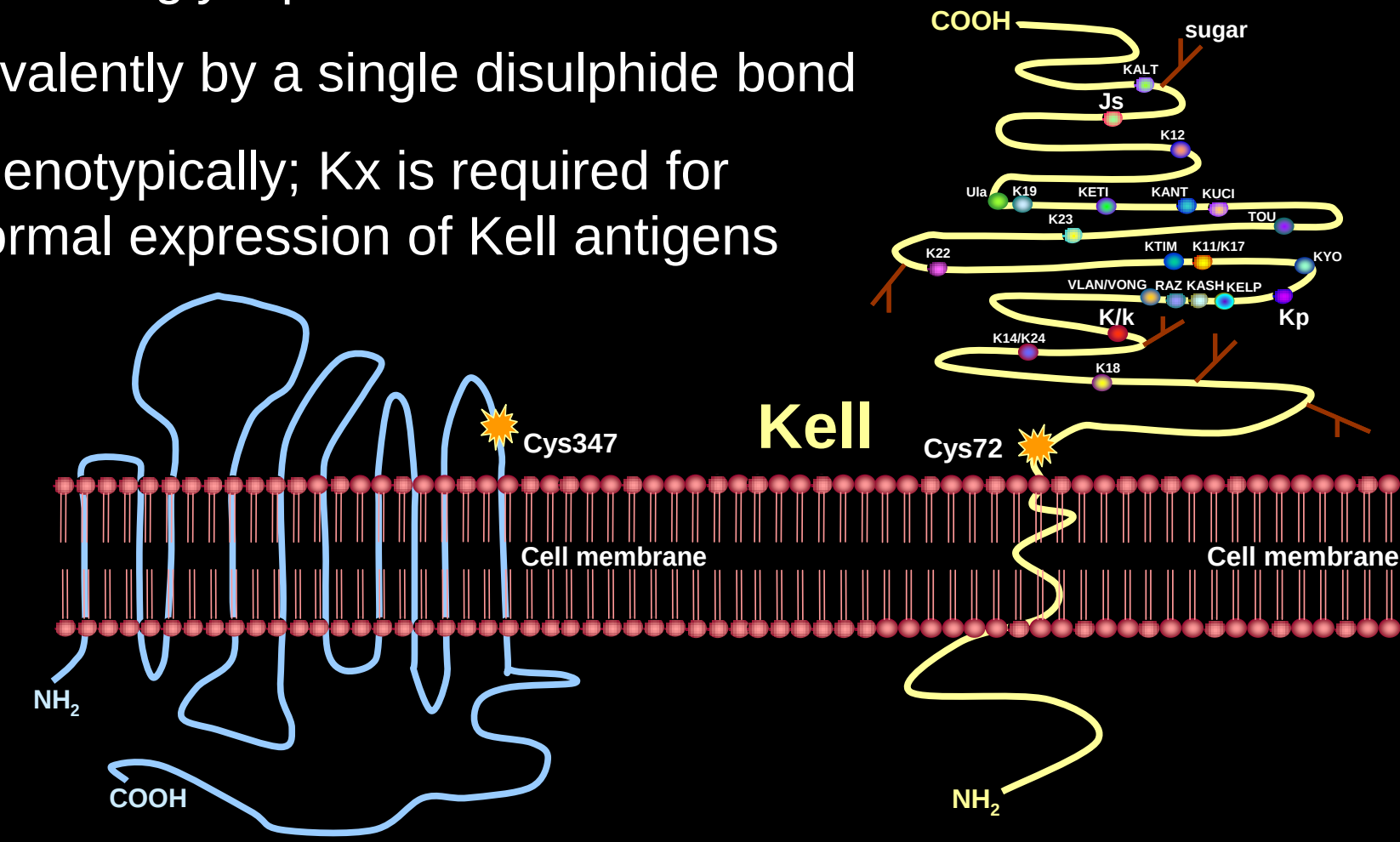


- Predominantly expressed in erythrocytes

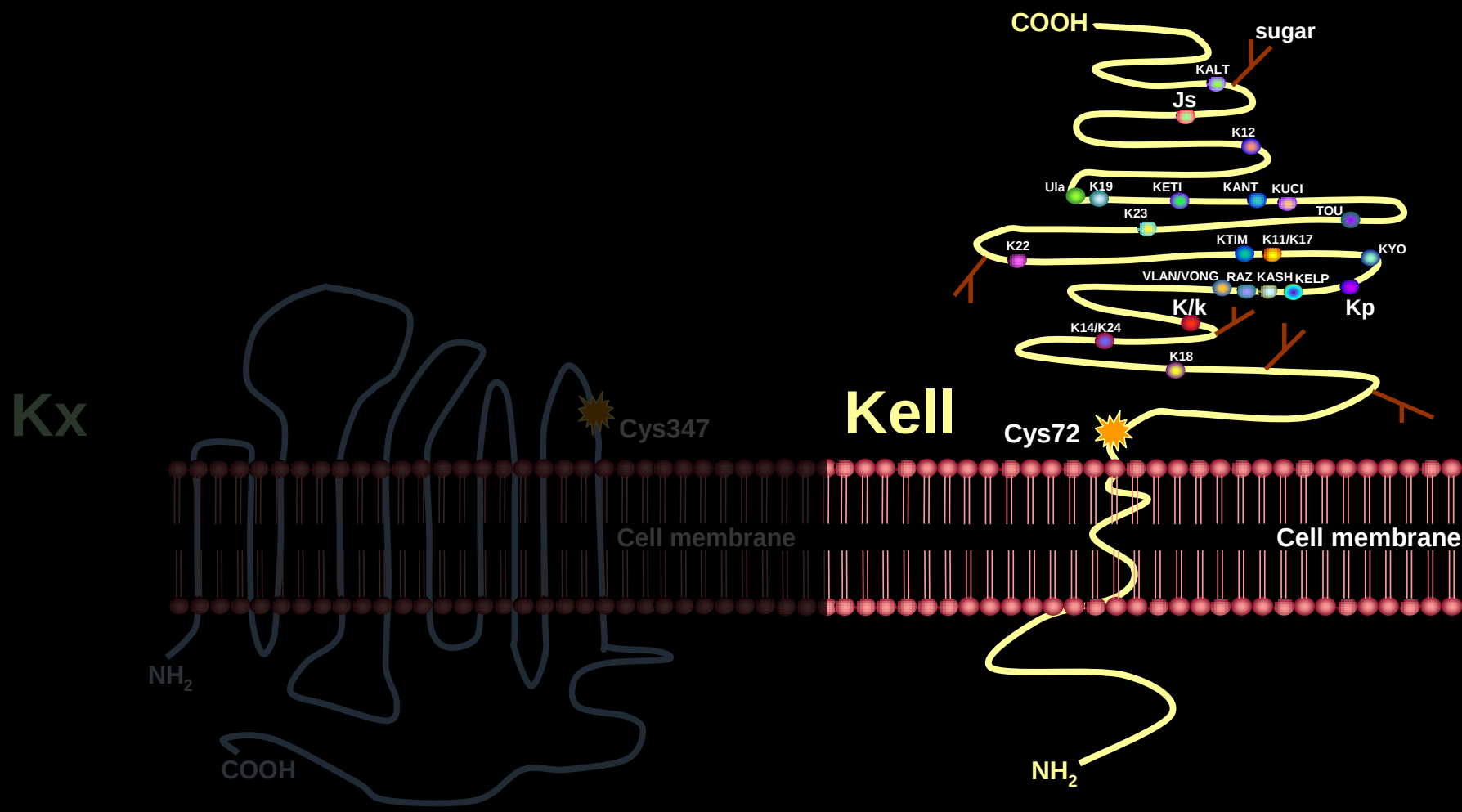
Kx and Kell interaction

Kx and Kell glycoprotein are linked:

- covalently by a single disulphide bond
- phenotypically; Kx is required for normal expression of Kell antigens



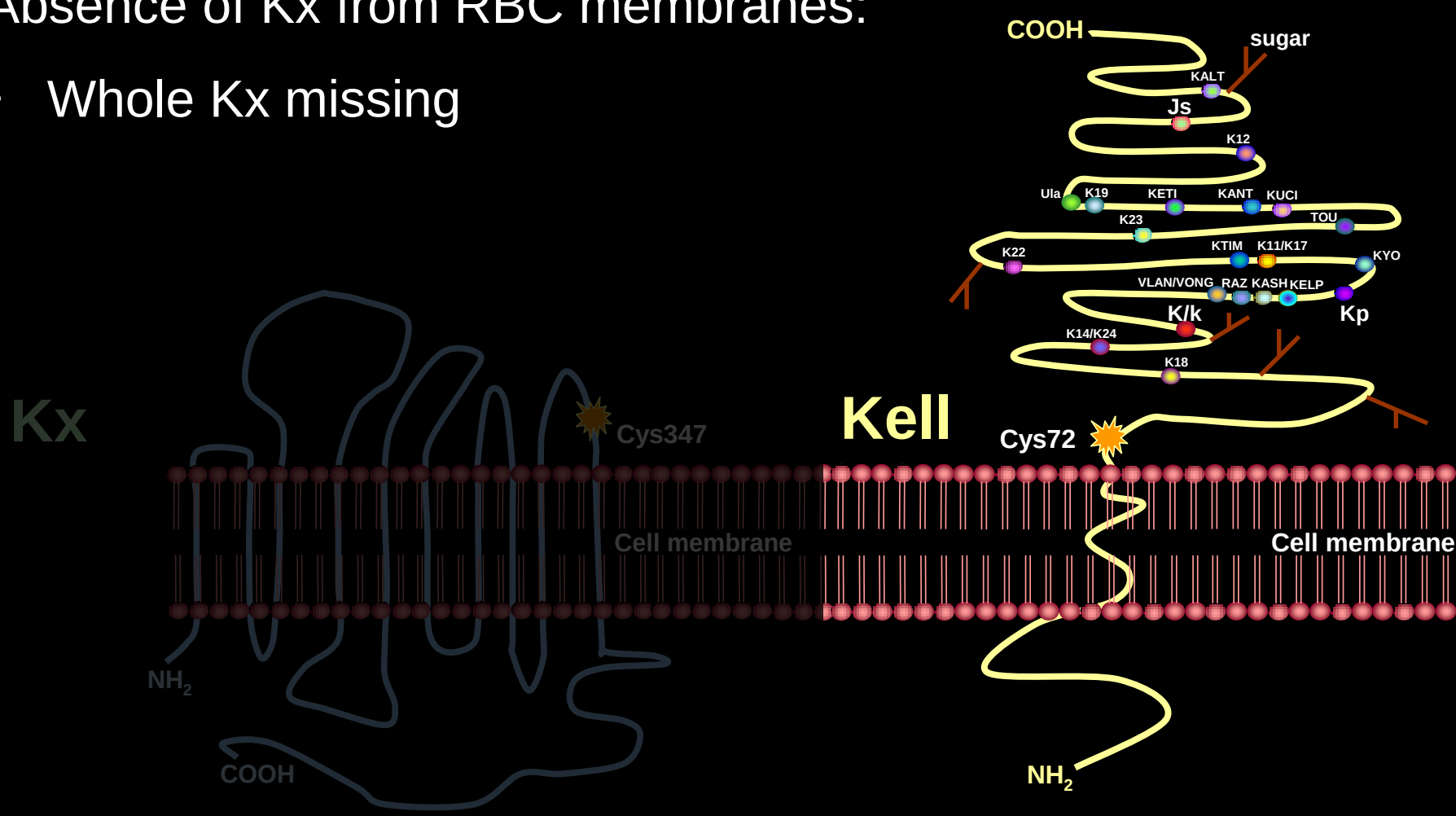
Kx and Kell interaction in MLS



Kx and Kell interaction in MLS

Absence of Kx from RBC membranes:

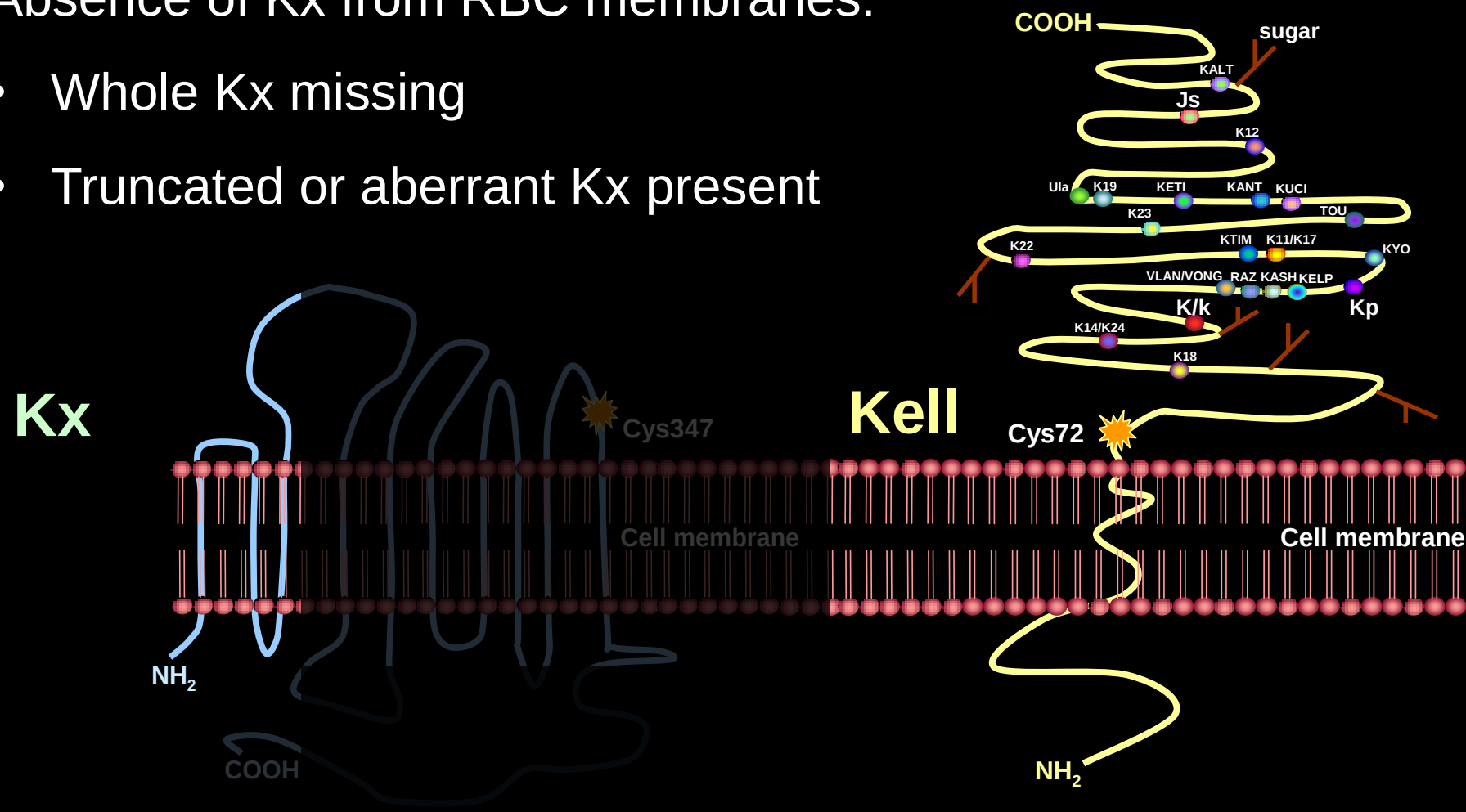
- Whole Kx missing



Kx and Kell interaction in MLS

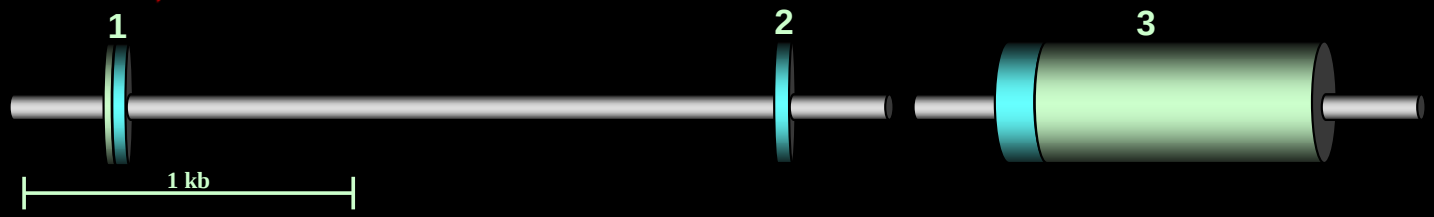
Absence of Kx from RBC membranes:

- Whole Kx missing
- Truncated or aberrant Kx present



XK gene

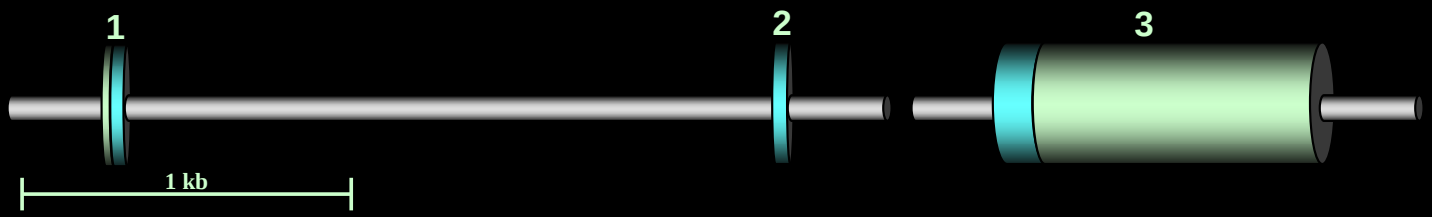
- Kx is encoded by X-linked gene, *XK*
- MLS is X-linked syndrome, predominantly affecting males



- Located at Xp21.1
- 46.2 kb in size
- Organised in 3 exons

MLS mutations in *XK* gene

- ISBT lists 29 *XK* inactivating genotypes
- They can be grouped in 5 categories



1. Deletion mutations
2. Single base insertion, reading frame shifts
3. Single base nonsense mutations
4. Single base missense mutations
5. Splice site mutations

Serology tests at IBGRL

Ab compatible with McLeod cells, weak with Ko cells

McLeod phenotype?

Patient's antibody confirmed:

- Compatible with several examples of McLeod cells and K_{mod} cells
- Weakly reactive with Ko cells
- Strongly incompatible with all other routine panel cells

Patient's cells confirmed:

- Reduced expression of all Kell antigens
- Compatible with anti-KL (anti-Km + anti-Kx)

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Serology tests at IBGRL

Test cells	Antiserum		
	-KL		-Proband
	-Kx	-Km	
McLeod	-	-	-
Ko	+++	-	w
Common	w	+++	+++
Proband	-	-	-

Serology tests at IBGRL

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Ko	+++	-	w
Common	+++	+++	+++
Proband	-	-	-

Patient's antibody appeared to be predominantly anti-Km with weak anti-Kx

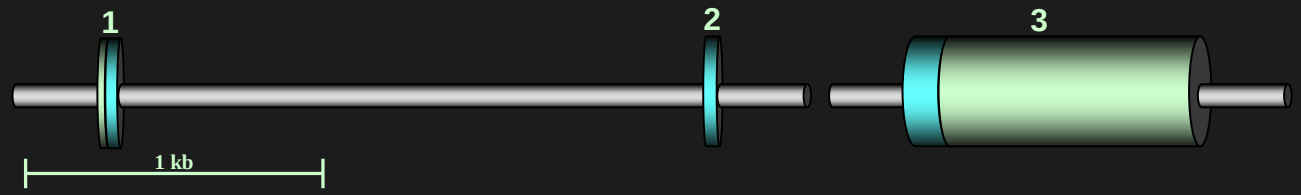
anti-Km + weak anti-Kx + anti-S

Genetics

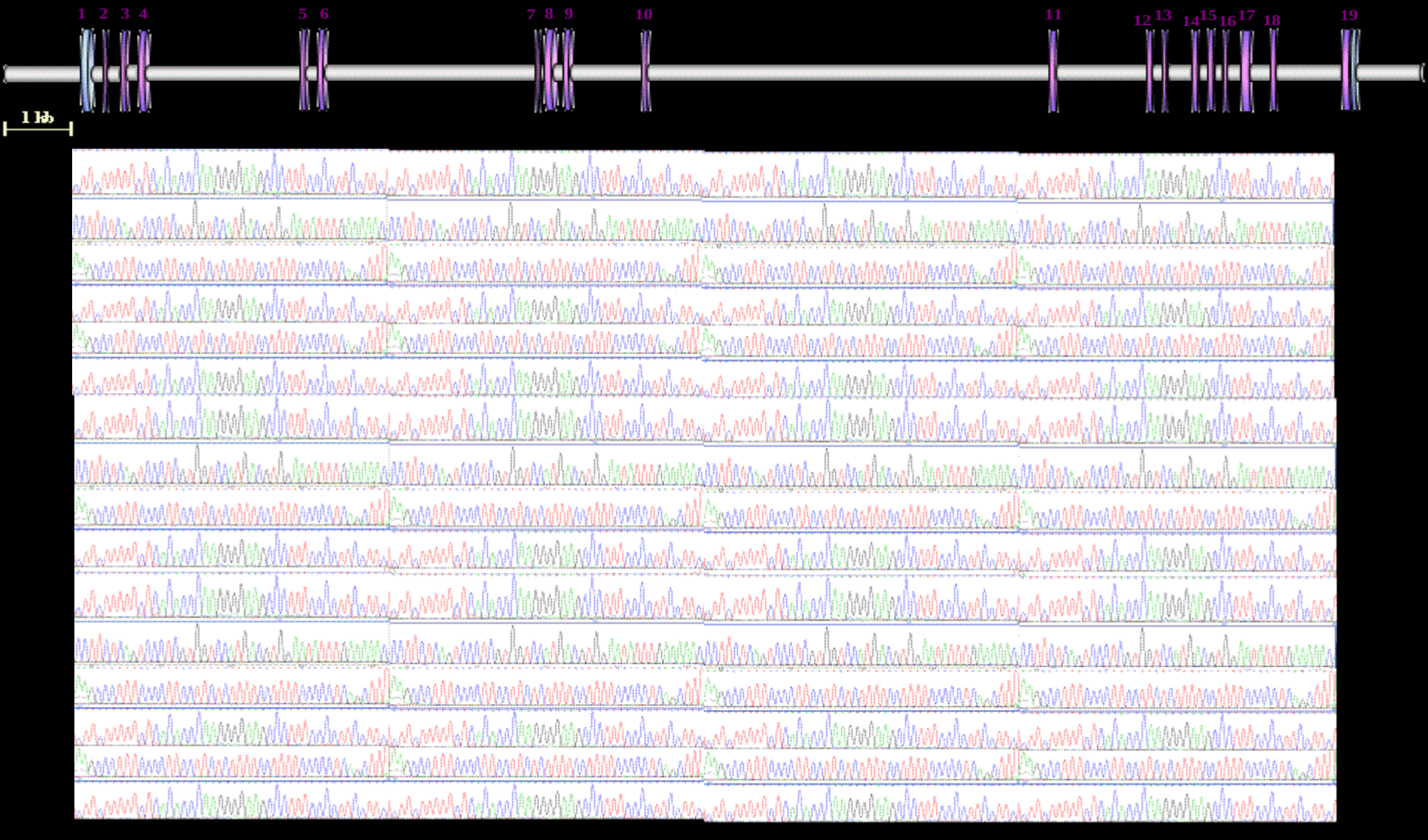
Proband referred for sequencing of:

KEL

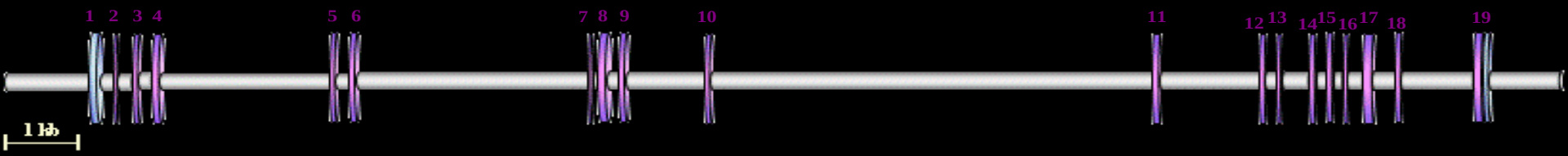
XK



KEL

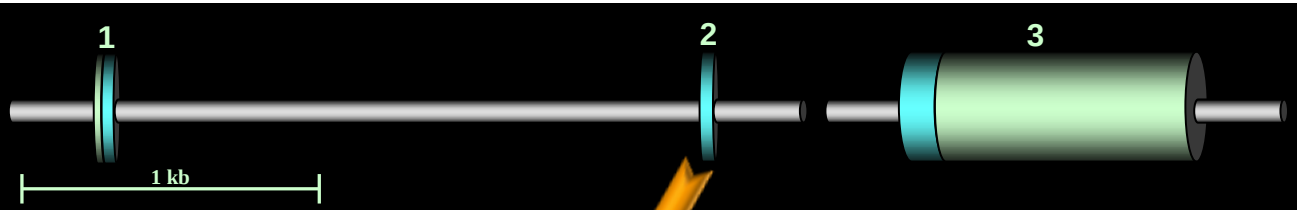


KEL

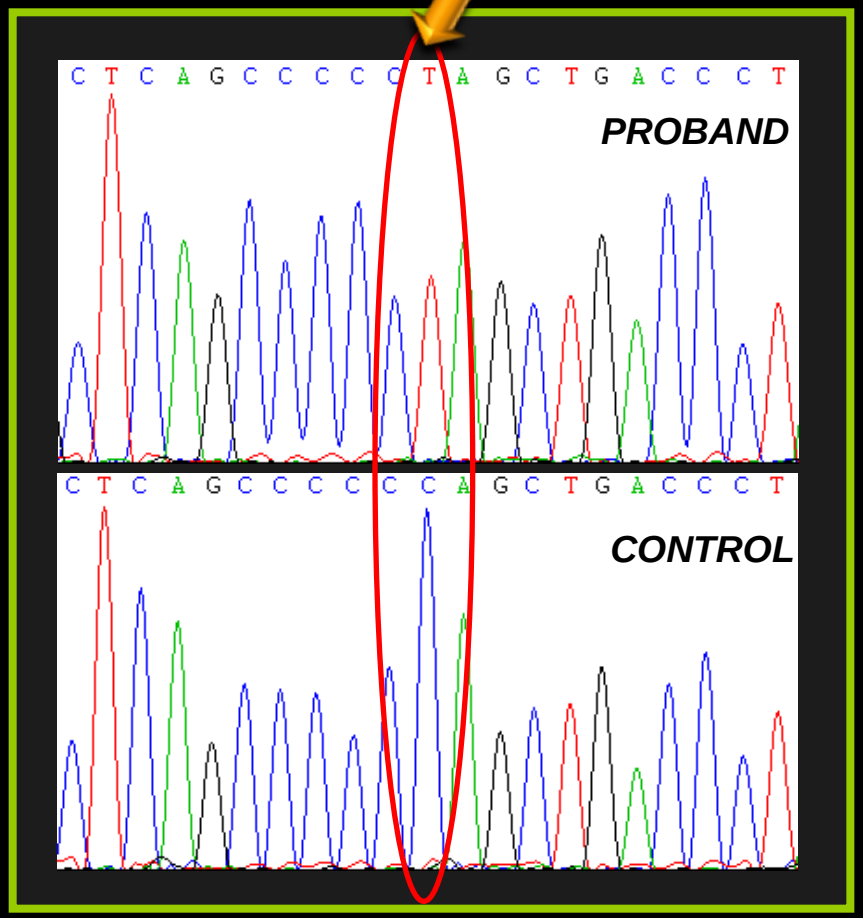


No mutations in the whole coding region of *KEL*; proband was of common *KEL2* genotype

XK



XK exon 2

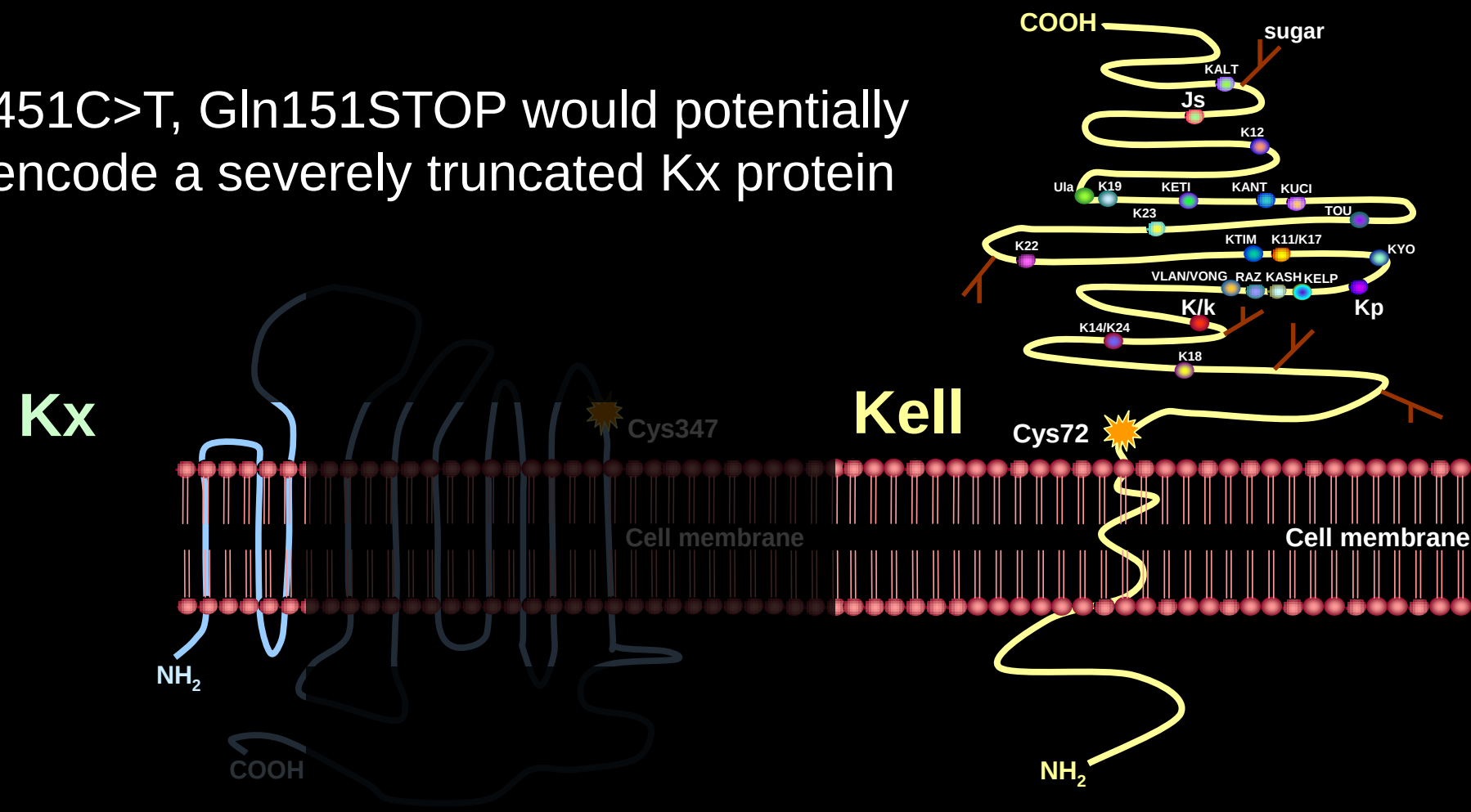


451C>T

Gln151STOP

XK mutation in the proband

451C>T, Gln151STOP would potentially encode a severely truncated Kx protein



Acanthocytosis and some neurological abnormalities were found

Proband’s family study

DNA from 10 family members sent for *XK* examination

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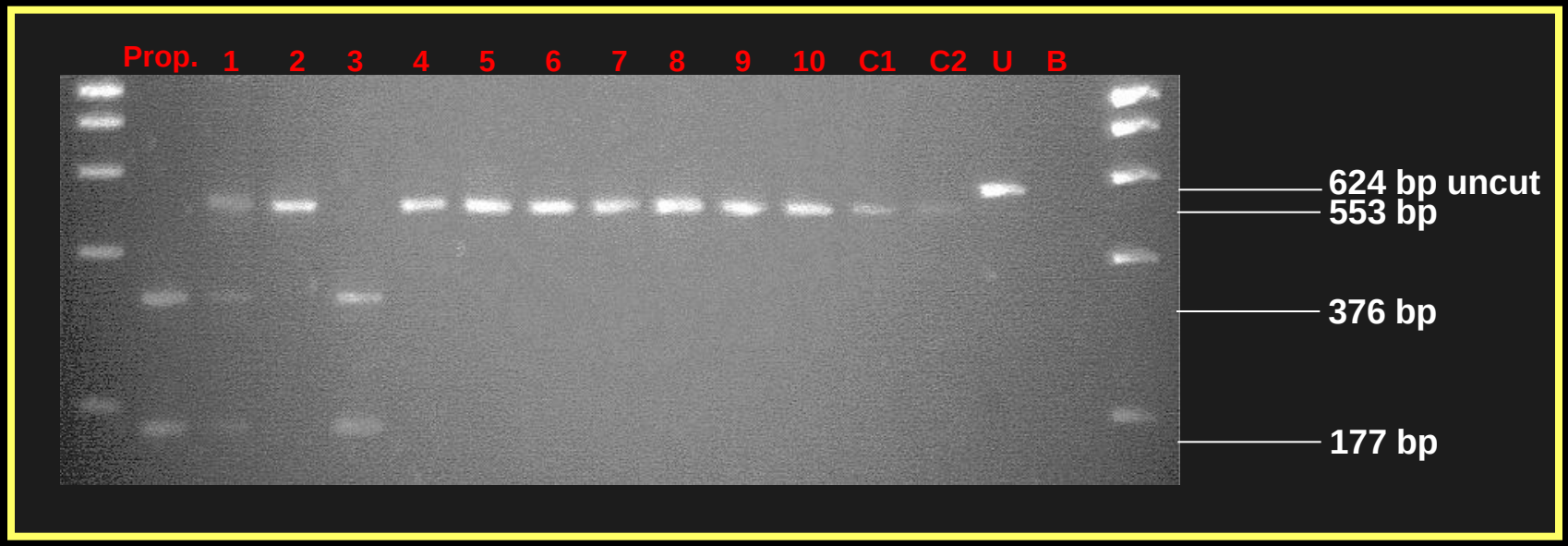
PCR-RFLP test for 451C>T mutation:



*Bfa*I cuts wild type 2x (553 + 63 + 8 bp)

mutation 3x (367 + 177 + 63 + 8 bp)

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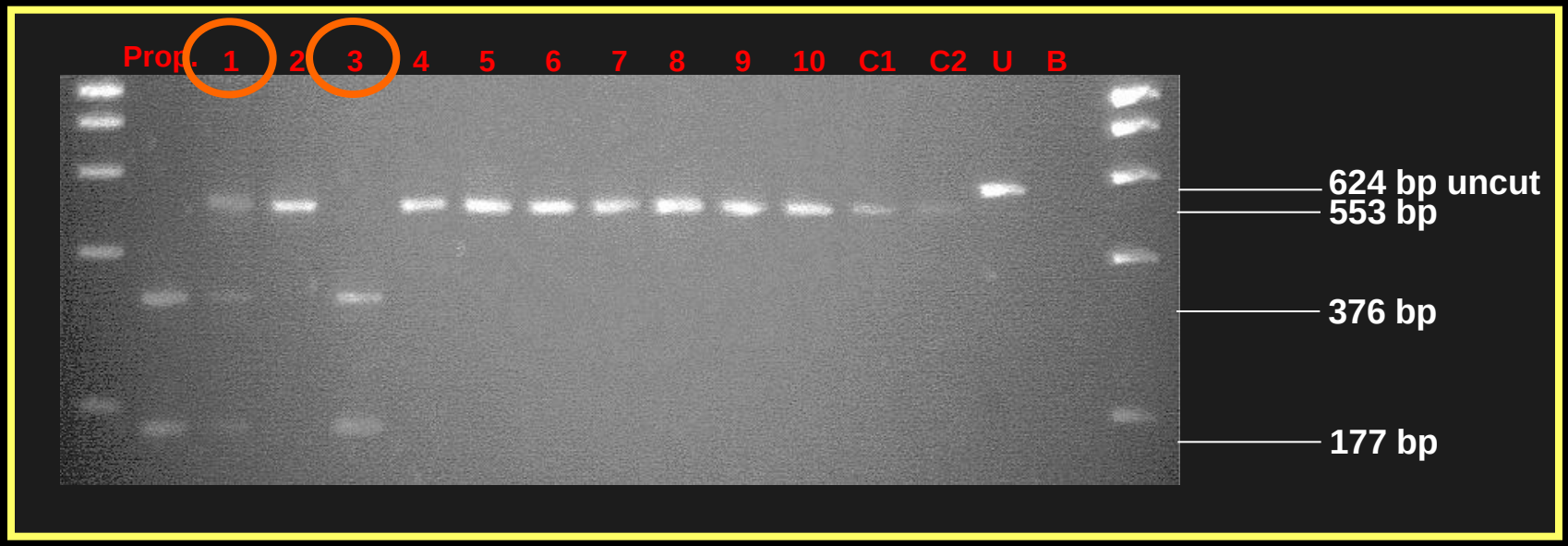

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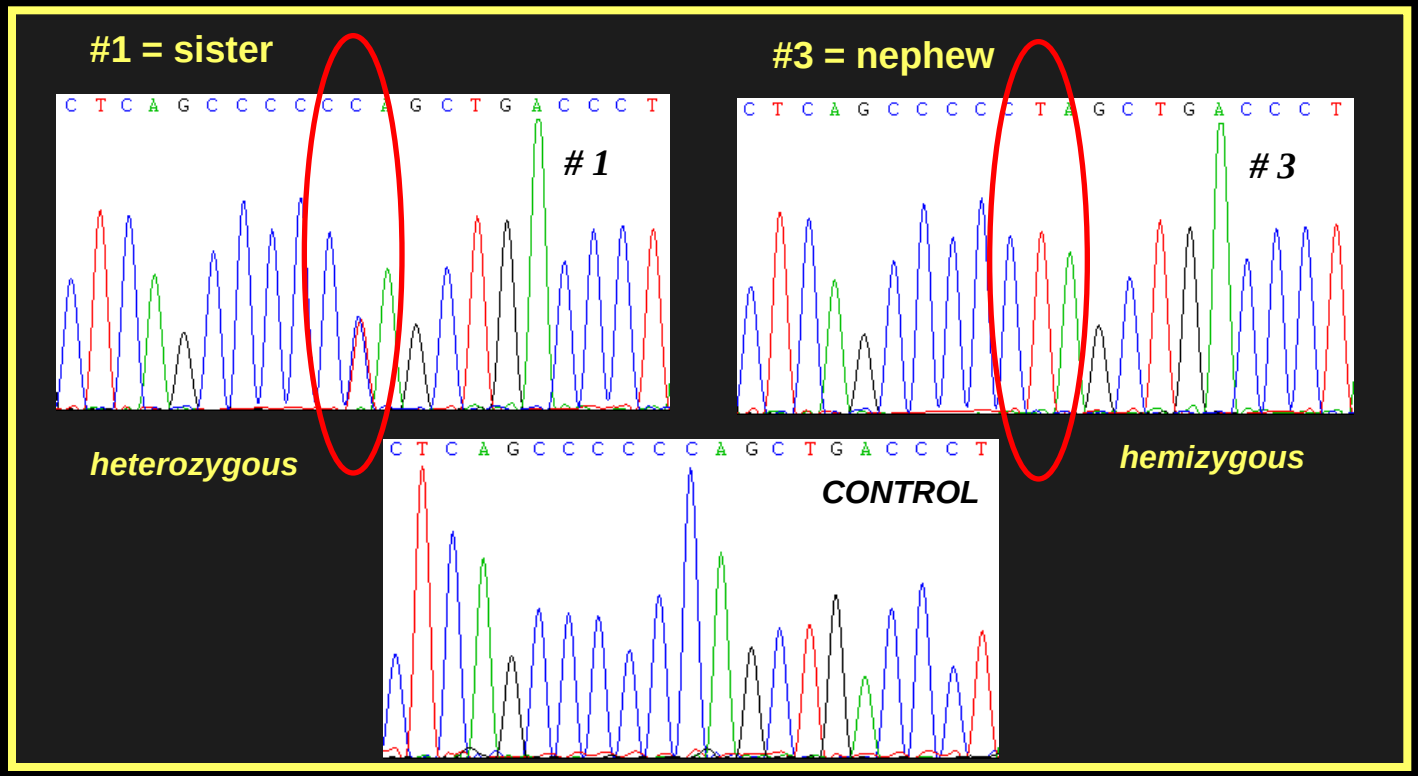
Sequencing of the whole *XK* coding region:

451C>T (Gln151STOP) in exon 2:



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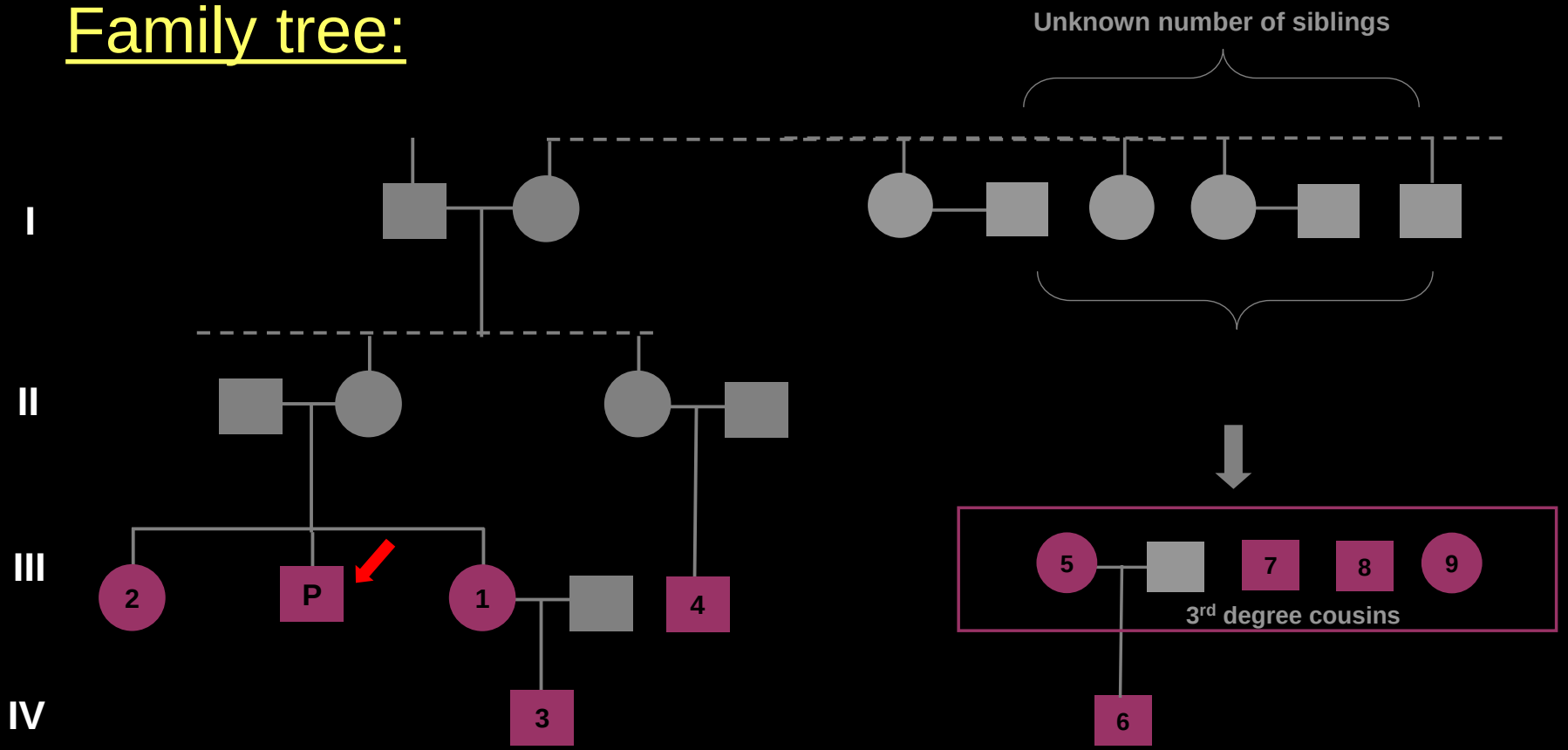
Family tree:



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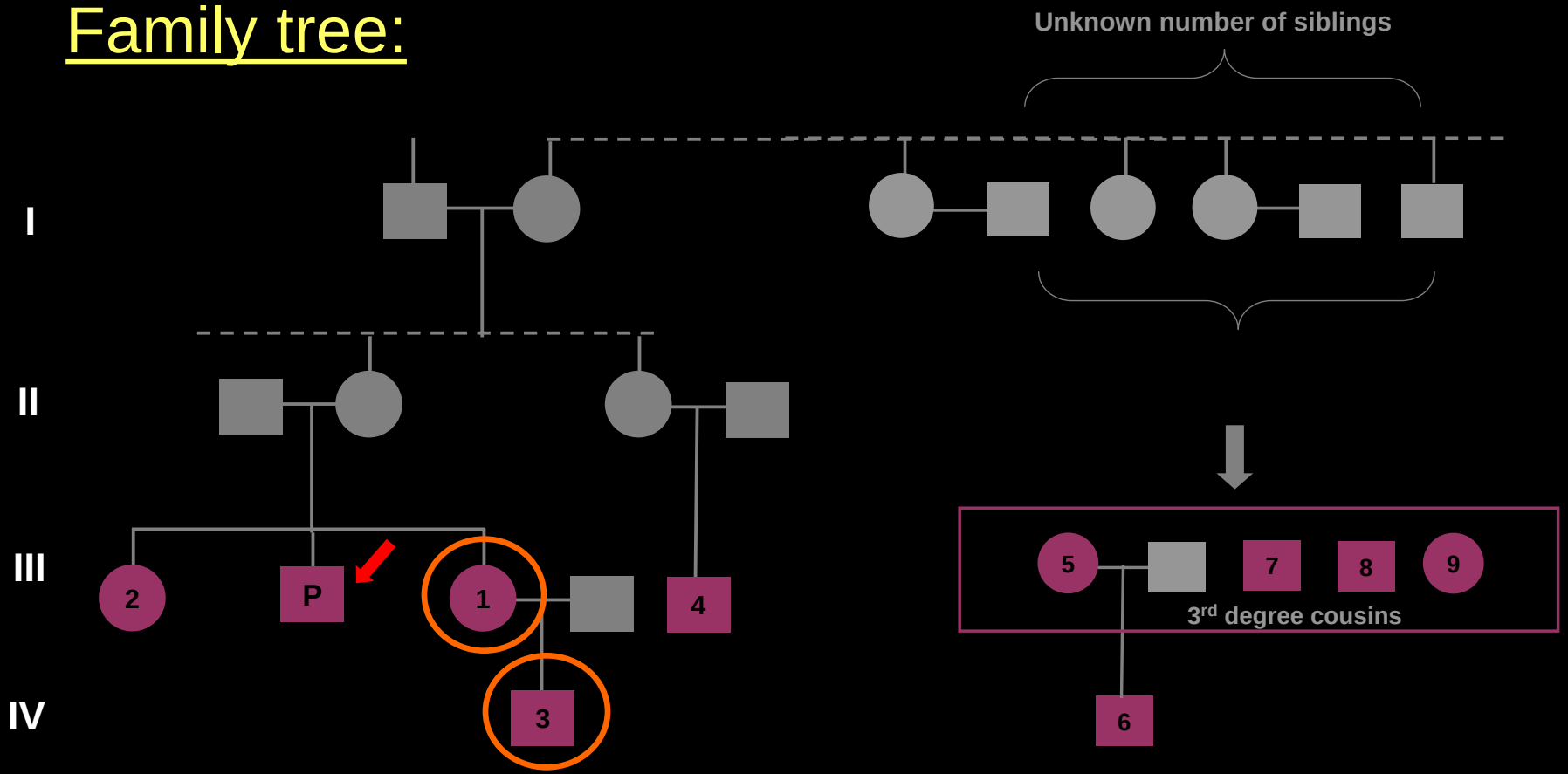

Conclusions

We have described:

- A novel inactivating mutation 451C>T, Gln151X, in *XK* of a patient with McLeod phenotype
- The patient's serum contained anti-Km+weak anti-Kx+ anti-S which were stimulated by one unit of crossmatch compatible blood following surgery
- Patient had acanthocytosis and some neurological abnormalities