BSH 2020 VIRTUAL
9 -14 NOVEMBER

British Society for Haematology
60 1960–2020
Listening • Learning • Leading

Improving the Laboratory Diagnosis of Pyruvate Kinase Deficiency

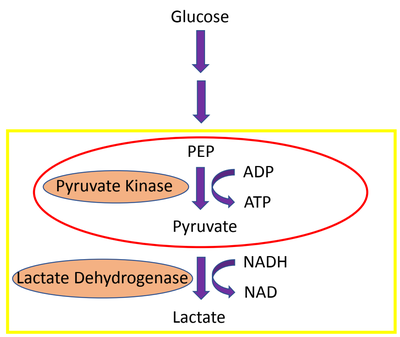
C. LAAS[1], C. LAMBERT[1], T. SENIOR MCKENZIE[1], D . REES[2] and B. CLARK[3]
1 Red Cell Centre, Viapath, King's College Hospital, London, UK
2 Department of Haematological Medicine, King's College Hospital, London, UK
3 Precision Medicine, King's College Hospital, London, UK

viapath
King's College Hospital NHS
NHS Foundation Trust

INTRODUCTION

Pyruvate kinase (PK) deficiency is an autosomal recessive disease caused by mutations in the *PKLR* gene, which reduce PK enzyme activity resulting in decreased energy synthesis in erythrocytes. This causes the red blood cells to break down prematurely, causing haemolytic anaemia.

Historically, the investigation into Pyruvate Kinase deficiency (PKD) has been led by a red cell enzyme assay. However, various factors are known to interfere with the assay, and multigenic causes (e.g. *KLF1* with *PKLR*) have been found to result in PKD.



Glycolysis pathway. The PK enzyme assay utilizes the reaction circled in red

The advent of genetic testing allows us to re-assess the reference range of the enzyme assay, and subsequently improve diagnosis of PKD.

AIM

- To test the lower end of the reference range to determine how effective it is at discriminating between affected and unaffected individuals using genetic analysis
- To generate a combined testing algorithm based on enzyme activity screening and genetic testing which minimises the number of false negative diagnoses
- Analyse the clinical significance of any novel variants found during the study

METHOD

- The *PKLR* gene was sequenced in 155 individuals, which included individuals suspected of having PKD and individuals identified as having normal haematology, to develop a truth set of confirmed positives (*sensitivity*) and confirmed negatives (*specificity*)
- MLPA analysis of *PKLR* and sequencing of the *KLF1* gene were performed on individuals with <2 pathogenic variants and a PK activity outside of the reference range (11-19 IU/g Hb)
- Published data and *in silico* prediction tools were used to analyse novel variants identified during the study
- To exclude bias of ages, sex and ethnicity when combining the dataset, anonymised patient data and IQC data from volunteers over time was audited for patients tested for PKD at King's College Hospital

RESULTS

Genetic and Protein Analysis

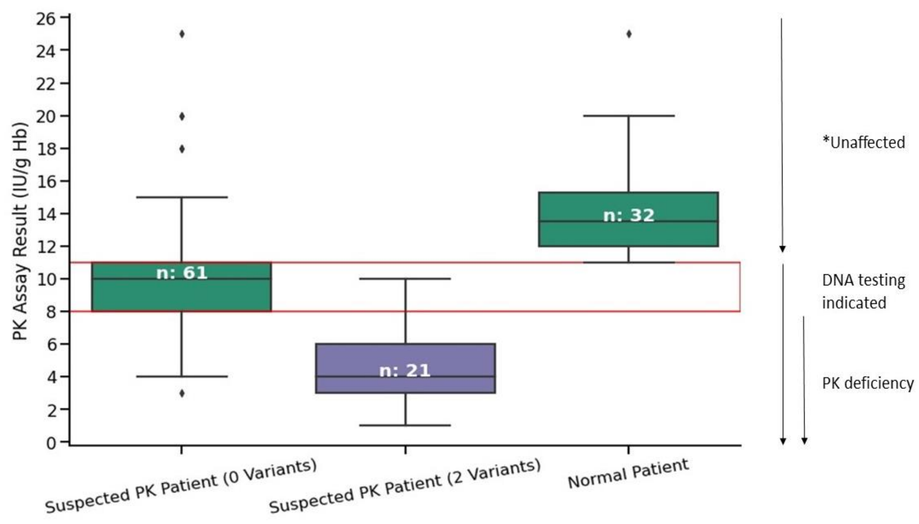
There is overlap between those with pathogenic variants and those with none or only one pathogenic variant found - **Figure 1**

- 0 variants - PK activity 7.0-53.4 IU/g Hb
- 1 pathogenic variant - 4.5-10.6 IU/g Hb
- ≥2 pathogenic variants - 1.3-9.1 IU/g Hb

The genetic results were used to assess the sensitivity and specificity of the PK assay - **Figure 2**

- The crossover point of sensitivity and specificity was 7.75 IU/g Hb (rounded up to 8.0)

Figure 1

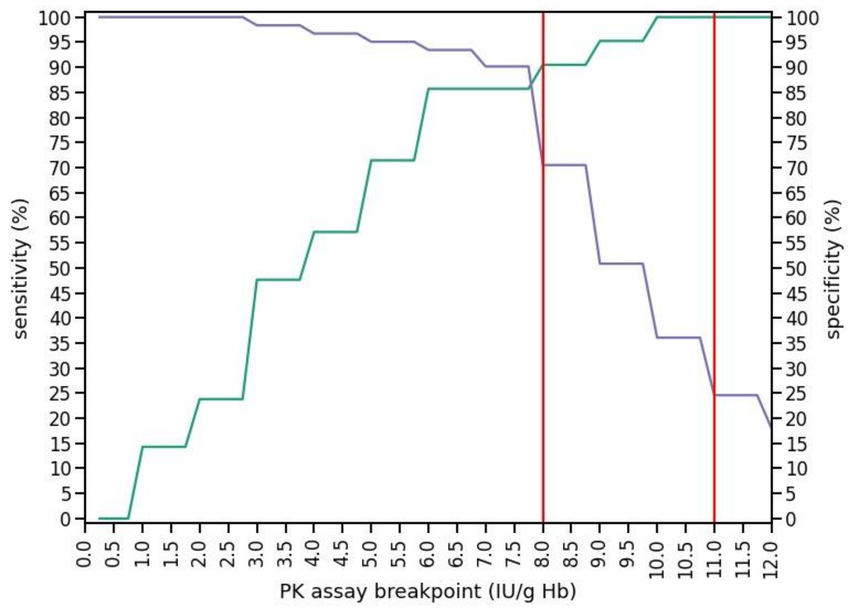


10 novel variants were found in the PKLR gene, 6 of which are likely to be clinically significant – **Table 1**

Table 1

Novel Variant		ACMG Score
DNA Change	AA Change	
c.319 A>G	p.107 M>V	4
c.508-2 A>G		4
c.508-57 G>A		2
c.627 G>C	p.209 R>R	2
c.822 C>G	p.274 F>L	3
c.959T>C	p.320 V>A	4
c.965+2 T>C		4
c.1076G>C	p.359 R>P	3
c.1299 C>A	p.433 Y>X	4
c.1437-131_c.1619-114 del1277		5

Figure 2



Audit of Patient and Control Data

- PK activity in males vs females: p=0.2441 - *not significant*
- PK activity African vs White European: p=0.9802 - *not significant*
- A **decrease** was **observed** in individual females over time, however there was insufficient data for robust statistical analysis

CONCLUSIONS

We defined a cut-off of 7.75-11 IU/g Hb, rounded off to 8.0-11.0 IU/g Hb. We used this with our test groups to define a new testing approach, where the *assay* is the *first-line* screen:

a) >11 then the individual is not deficient

b) ≤11 then genetic testing is required to confirm

c) <8 then the result is PK deficiency but genetic testing would be useful to confirm and identify the mutation for other purposes e.g. prenatal diagnosis

The results also indicate there is no *significant* difference in PK activity for gender, age and ethnic origin, although greater numbers are needed to confirm.

ACKNOWLEDGEMENT

Thank you to Dr Barnaby Clark of King's Health Partners for his support and guidance.

The laboratories of the Red Cell Centre of Viapath Analytics at King's College Hospital for their assistance and resources.

Philip Davidson and Ewart Sheldon of King's Health Partners for their assistance in data collection and arrangement of data and figures.

REFERENCES

- **Beutler E. *et al.*** International Committee for Standardization in Haematology: Recommended Methods for Red-Cell Enzyme Analysis. *Br J Haematol 1977;* 35; 331-339.
- **Lezon-Geyda K. *et al.*** *PKLR* Intron Splicing-Associated Mutations and Alternate Diagnoses Are Common in Pyruvate Kinase Deficient Patients with Single or No *PKLR* Coding Mutations. Poster presentation given at the "European Haematology Association Annual Congress" conference hosted by the European Haematology Association on June 14-17, 2018 at the Stockholmsmässan of Stockholm, Sweden [unpublished]
- **Zanella A., Bianchi P. and Fermo E.** Pyruvate kinase deficiency. *Haematologica 2007;* 92(6); 721-722.
- **Kung, C., *et al.*** AG-348 enhances pyruvate kinase activity in red blood cells from patients with pyruvate kinase deficiency. *Blood* 2017; 130(11); 1347-1356.

CONTACT INFORMATION

E-mail:
claire.laas@nhs.net barnaby.clark@nhs.net

Correspondence: Barnaby Clark, Precision Medicine, 1st Floor Bessemer Wing, King's College Hospital, Denmark Hill, London SE5 9RS