Introduction to Pyruvate Kinase Deficiency (PKD)

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Department of Clinical Chemistry and Haematology
This talk is applicable for:

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<thead>
<tr>
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<th>Definite</th>
<th>Probable</th>
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<tbody>
<tr>
<td>Thalassemia</td>
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<td>Sickle cell disease</td>
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<td>Membrane disorders (e.g. spherocytosis)</td>
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<td>Enzyme defects (e.g. PKD, G6PD)</td>
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<td>PNH</td>
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<td>Other forms of hemolytic disease</td>
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### Disclosures

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<tr>
<th>Company name</th>
<th>Research support</th>
<th>Employee</th>
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<th>Stockholder</th>
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<td>Agios Pharmaceuticals</td>
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PKD is a hereditary **chronic hemolytic anaemia**

**Prominent clinical features**

- Anaemia (highly variable)
- Exacerbation of anaemia by infection
- Splenomegaly
- Jaundice / gall stones
- Iron overload (also in non-transfused patients)
The red blood cell

Stem cell → BFU-E → CFU-E → Colony formation (in vitro) → Pro-erythroblast → Basophilic erythroblast → Polychromatic erythroblast → Orthochromatic erythroblast → Reticulocyte → Erythrocyte

Bone Marrow

Blood stream

EPO

Nuclear extrusion
The red blood cell

- Highly differentiated cell, no organelles
- Survives 110-120 days
- Efficient transport of $O_2$ and $CO_2$
- Large surface-to-volume area
Proper red cell function requires energy

Cellular metabolism:

- Keeps iron of haemoglobin in the functional, ferrous ($\text{Fe}^{2+}$) form
- Maintains intracellular potassium (high), sodium and calcium (low) levels
- Keeps protein sulhydryl groups (enzymes, hemoglobin, membrane proteins) reduced
- Maintains the red cell’s biconcave shape
Red blood cell metabolism

Embden-Meyerhof pathway

Hexose Monophosphate Shunt

Glutathione pathway

Rapoport-Luebering Shunt

Nucleotide metabolism

phosphoenolpyruvate
ADP
ATP
Pyruvate kinase

pyruvate
Metabolic consequences of PKD

PK enzymatic activity decreased

- **Depletion** of ATP (ATPase activity decreased – calcium influx?)

- **Accumulation** of 2,3-bisphosphoglycerate
  - Suppression HMP shunt – susceptibility to oxidative stress
  - $O_2$ more readily delivered to tissue

- **Accumulation** of glucose-6-phosphate (inhibition hexokinase – EMP)
Lack of ATP leads to extravascular hemolysis

- Energy deprivation
- Metabolic depletion /accumulation
- Altered properties
- Premature removal from the circulation by the spleen

6th European Symposium on Rare Anaemias - 1st Dutch-Belgian meeting for patients and health professionals
Lack of ATP leads to extravascular hemolysis

- Low pH
- Low glucose
- Low oxygen
- High oxidant
- Macrophages

Mebius and Kraal. Nat Rev Immunol, 2005

Anemia, splenomegaly, jaundice
Lack of ATP leads to extravascular hemolysis

- Low pH
- Low glucose
- Low oxygen
- High oxidant
- Macrophages

PKD: selective destruction of PK-deficient reticulocytes!

Mebius and Kraal. Nat Rev Immunol, 2005
Lack of ATP leads to extravascular hemolysis

- Energy deprivation
- Metabolic depletion /accumulation
- Altered properties / deformability
- Premature removal from the circulation by the spleen
Red blood cell deformability

Elongation index

Osmolarity (mOsmol/L)

Huisjes et al, unpublished
Blocking glycolysis decreases cell deformability

Huisjes et al, unpublished
PKD patient cells show loss of deformability

Huisjes et al, unpublished
PKD cells show decreased deformability upon exposure to oxidative stress

Van Wijk et al., unpublished

Exacerbation of anemia by infection (?)
Ineffective erythropoiesis contributes to the pathogenesis of PKD

- Increased numbers of hematopoietic precursors, *e.g.* BFU-E
- Increased apoptosis in spleen cells from PK-deficient mice and a human patient

Aizawa et al.
Ineffective erythropoiesis contributes to the pathogenesis of PKD...

- Spontaneous apoptosis in SLC3 (murine PK-R-deficient Friend erythroleukemic cell line)

- PK-R overexpression:
  - Downregulation of proapoptotic genes (e.g. Bad, Bnip3, Bnip3i)
  - Downregulation of antioxidant genes (e.g. Prdx1, Cat)

...and may contribute to iron overload in PKD

- Decreased levels of hepcidin, increased levels of GDF15 (as in β-thalassemia)

PKD is a **hereditary** chronic hemolytic anaemia

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Red blood cell PK is encoded by *PKLR*

- Located on chromosome 1q21
- 12 exons, 9.5 kb in size
- Encodes both PK-R and PK-L (tissue-specific expression)
- PK-R cDNA is 2060 bp
Red blood cell PK is encoded by *PKLR*

- PK-R monomer is comprised of 574 amino acids
- 63 kDa
- N, A, B, C domain

van Wijk *et al*. Hum Mutat (2009)
Red blood cell PK is encoded by *PKLR*

- 250 kDa homotetramer
- Active enzyme

van Solinge and van Wijk. Williams Hematology (in press)
Mutations in *PKLR* cause PKD

PKD is an autosomal recessive disease

- Estimated prevalence: 51 cases per million (general white population)
- Carrier frequency 1:20,000

However

- 41 compound heterozygous/homozygous patients in NL
- Carrier frequency 10-20 times lower?
Mutations in *PKLR* cause PKD

- 249 mutations (as of November 2015)
- Almost all mutations also affect the PK-L isoform!

Van Wijk et al, Blood (2005)
Clinically, *PKLR* mutations affect only the red blood cell (?)

Pyruvate kinase deficiency associated with severe liver dysfunction in the newborn

Martine F. Raphaël,¹* Richard Van Wijk,² Joachim J. Schweizer,³ Netteke A.Y. Schouten-van Meeteren,⁴ Angelika Kindermann,⁵ Wouter W. van Solinge,² and Frans J. Smiers⁶

Am J Hematol (2007)

Cholestasis and Hepatic Failure in a Neonate: A Case Report of Severe Pyruvate Kinase Deficiency

François Olivier, MD⁷, Anna Wieckowska, MD⁷, Bruno Piedboeuf, MD, FRCP⁷, Fernando Alvarez, MD, FRCP⁷

Pediatrics, 2015

Catherine Vezina, personal communication (july, 2015)
Mutations in *PKLR* cause PKD

- 249 mutations (as of November 2015)
- Mostly point mutations
- Few recurrent mutations (*e.g.* c.1529G>A, c.1456C>T)
- 75% of mutations are missense mutations

*Van Wijk et al, Blood (2005)*
Genotype-to-phenotype correlation in PKD

c.331G>A, p.Gly111Arg (PK Utrecht)
severe sterical hindrance

Van Wijk et al. Hum Mutat (2009)
Genotype-to-phenotype correlation in PKD

- Determined by location of affected residue, and nature of substitution
- Enzyme activity (catalytic efficiency)
- Regulatory properties (modulation by FBP/ATP)
- Protein stability (i.e. mutations affecting the subunit interface)
- Most patients are compound heterozygous!

Van Wijk et al. Hum Mutat (2009)
Therapy

“Classical” therapy
- Blood transfusion
- Splenectomy
- Iron chelation
- (Stem cell transplantation)

New therapeutic approaches
- Activator treatment (Agios Pharmaceuticals Inc.)
- Gene therapy (ForGeTPKD, Garate et al. Stem Cell Rep 2015)
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Pavla Koralkova
Tesy Merkx
Brigitte van Oirschot
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Department of Medical Genetics
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