

Congenitale nicht-sphärozytische hämolytische Anämie – eine Ursachenforschung



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Problem



- Angeborene transfusionsabhängige normochrome, normozytäre, Coombs-negative hämolytische Anämie
- Negative Familienanamnese
- Keine ausreichende Diagnostik vor Beginn der Transfusionstherapie

Fall: 2 Jahre alte Patientin



- 2. Kind österreichischer, nicht konsanguiner Eltern, unauffällige Schwangerschaft
- 40. SSW, Spontangeburt, Geb. Gew. 4035g
- Ikterus gravis neonatorum: Unkonj. Bili 21.6 mg/dl
Coombs und Rh- Inkompatibilität neg.
- Austauschtransfusion 5 h postpartal
- Phototherapie
- Pfortaderthrombose





- Hb-Elektrophorese und Hämoglobinopathie - Genetik negativ
- FACS –5 ME Messung und Erythrozyten-Schlüsselenzyme nicht konklusiv da posttransfusionell
- Eltern: Hämoglobin und Reti im Normbereich

Pyruvatkinase Aktivität der Patientin und der Eltern

Analysis Parameters, Glycolytic Enzyme Activities, and Flow Cytometry

Patients and Their Parents

	Hb (g/dl)	Retic (2–28‰) ^a	Total bilirubin (0.2–1.5 mg/dl) ^a	Haptoglobin (30–200 mg/dl) ^a	PK (10–20 IE/gHb) ^a	G6PD (9–14 IE/gHb) ^a	EMA test ^b
Patient 1	7.8	50	4.0	<10	10.1	33.3	Normal
Mother 1	12.9	12	Nd	Nd	11	12.4	Nd
Father 1	15.8	15	Nd	Nd	6.4; 7.4	10.2	Nd
Patient 2							Normal
Mother 2							Normal
Father 2							Nd

G6PD, glucose-6-phosphate dehydrogenase; Hb, hemoglobin; Nd, not determined; PK, pyruvate kinase; Retic, reticulocytes. ^aPK and G6PD values were determined in the same laboratory. ^bEMA test was performed as normal.

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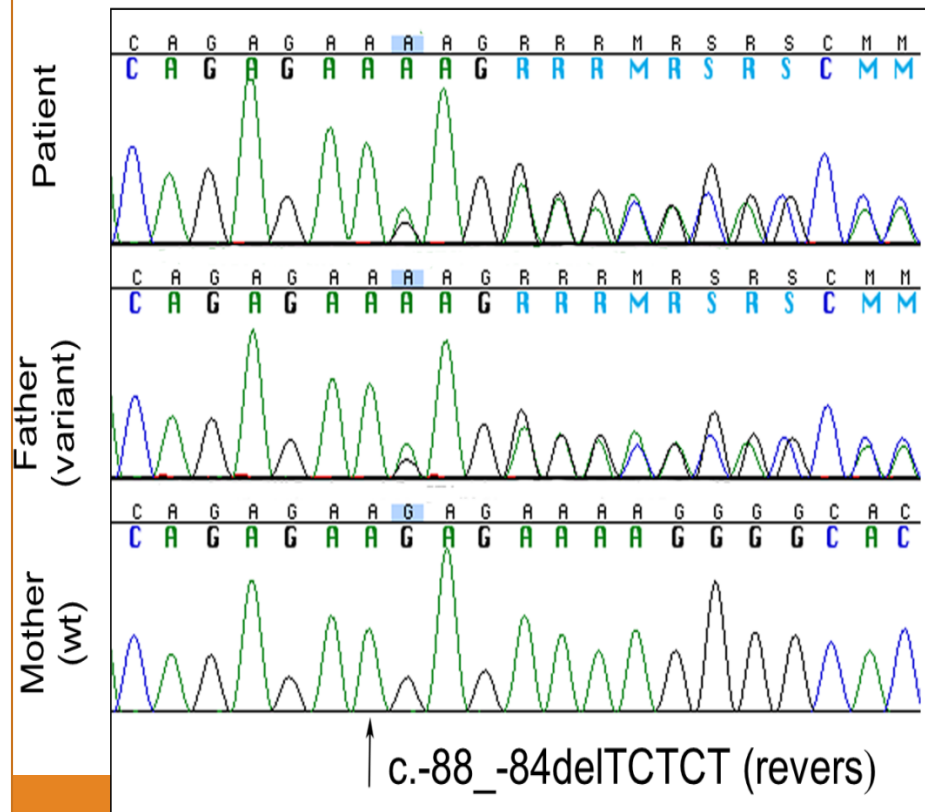
Patient 2 were performed in another laboratory (with different reference values).

Two Novel Missense Mutations and a 5bp Deletion in the Erythroid-Specific Promoter of the *PKLR* Gene in Two Unrelated Patients With Pyruvate Kinase Deficient Transfusion-Dependent Chronic Nonspherocytic Hemolytic Anemia

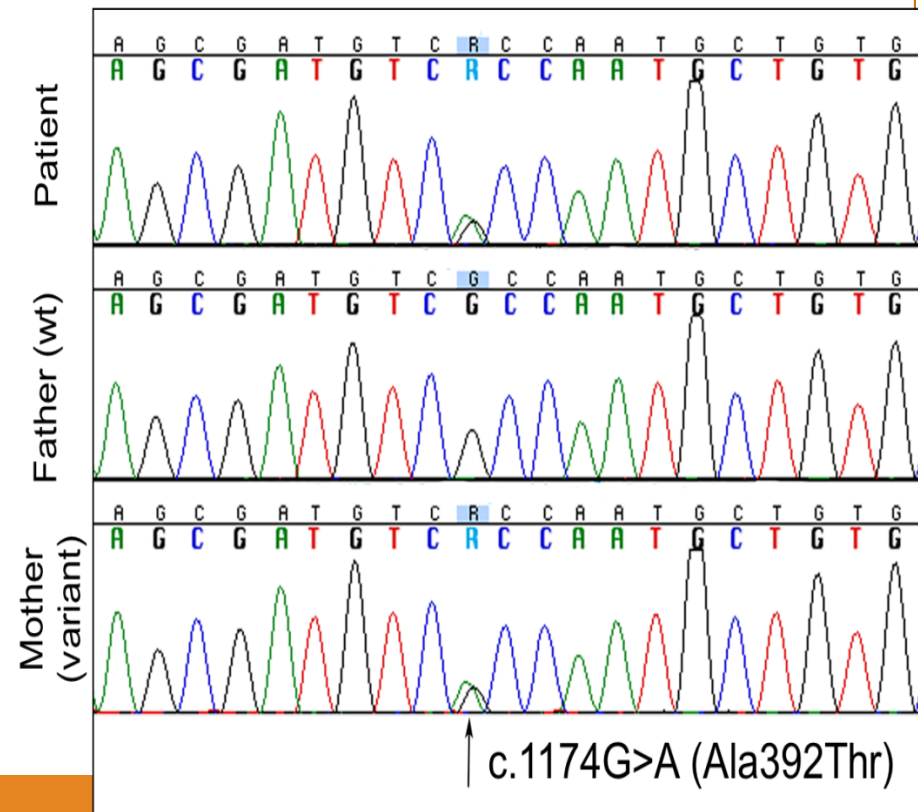
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Patient 1 (Girl)

5bp deletion in the promoter region
(c.-88_-84delTCTCT)



exon 9 (c.1174G>A; p.Ala392Thr)
missense mutation



5bp Deletion:
c.-88_-84delTCTCT

-119 ggggtggcctactgggtgtgccccttttctcttctctcccttcgata

PKR-RE1

-69 agaccagcagttttgtcatcctctccctctcattccatgggtcccgcagc

-19 ccaggcccacactgaaagc

-83G>C

-72A>G

Pyruvatkinasemangel



- Erstbeschreibung 1961. Häufigste Ursache einer angeborenen, nicht-sphärozytischen hämolytischen Anämie und häufigster Glycolysedefekt
- Prävalenz: 1:20.000
- Autosomal rezessiv; homozygot /compound heterozygot
- >240 Mutationen PKLR Gen
- *Variables klinisches Bild:*
 - Hydrops fetalis
 - Hyperbilirubinämie mit Austauschtransfusion
 - Transfusionsbedürftige chronische Hämolyse
 - Moderate bei Infekten gesteigerte Hämolyse
 - Stabilisierung im Erwachsenenalter

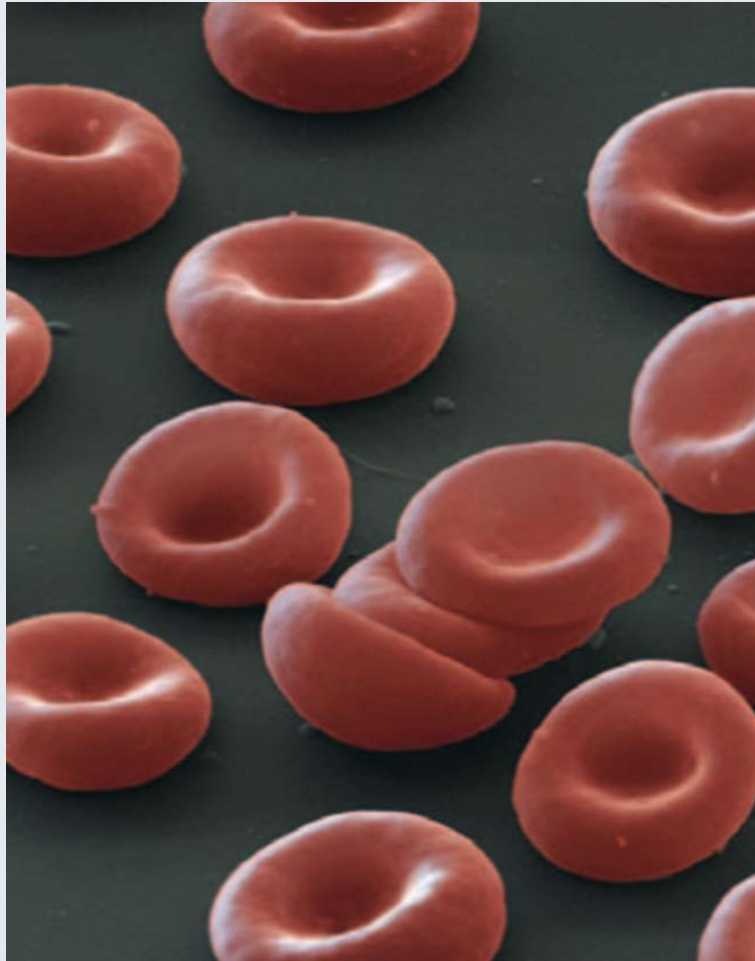
Diagnose PKD



- *PK-Enzymaktivität = Goldstandard*
- Falsch normal: Retikulozytose, Posttransfusionell
- PK Aktivität im Vergleich mit anderen Erythrozytenenzymen
- Sequenzierung PKLR-Gen
- des Patienten: limitierend bei compound heterozygotem Befund, großen Deletionen oder Intron-Mutationen
- Analyse der Eltern



- *Transfusionstherapie:*
 - Chronisch : Nadir bei Hb 7-9 mg/dl
 - Intermittierend
- *Splenektomie:* Hb Anstieg 1-3 mg/dl, Nutzen/Risiko
- Supportiv: Folsäure, Chelattherapie
- Impfungen, Penizillinprophylaxe
- Pharmakologischer PK-Aktivator AG-348



Embden-Meyerhof pathway

glucose → (ATP → ADP, Hexokinase) → glucose-6-phosphate → (NAD⁺ → NADH, Glucose-6-phosphate dehydrogenase) → 6-phosphogluconolactone → (NADP⁺ → NADPH, 6-phosphogluconolactonase) → 6-phosphogluconate → (NADP⁺ → NADPH, 6-phosphogluconate dehydratase) → ribulose-5-phosphate

Glutathione pathway

ribose-5-phosphate → (NADPH → NADP⁺, Glutathione reductase) → reduced glutathione (R-GS) ↔ (NADPH → NADP⁺) ↔ oxidized glutathione (GS-GS) → (R-GS → R-O-OH, peroxidase) → R-O-OH

Rapoport-Luebering Shunt

6-phosphogluconate → (phosphatase) → 6-phosphoglycerate → (kinase) → 1,3-bisphosphoglycerate → (Bisphosphoglycerate mutase) → 2-phosphoglycerate

Nucleotide metabolism

adenine → (Adenine phosphoribosyltransferase) → AMP → (Adenosine monophosphatase) → adenosine → (Adenosine deaminase) → inosine

adenosine → (Adenylate kinase) → AMP ↔ (ADP ↔ ATP) ↔ AMP → (Adenosine kinase) → adenosine

adenosine → (5'-nucleotidase) → 5'-ribonucleotide → (ribonucleoside) → ribonucleoside

Glycolysis and Gluconeogenesis

2-phosphoglycerate → (Enolase) → phosphoenolpyruvate → (ATP → ADP, Pyruvate kinase) → pyruvate → (NAD⁺ → NADH, Lactate dehydrogenase) → lactate

Other Pathways

phosphoenolpyruvate → (phosphatase) → 3-phosphoglycerate → (kinase) → 1,3-bisphosphoglycerate → (Bisphosphoglycerate phosphatase) → 2-phosphoglycerate