Newborn Screening for Cystinosis
&
the effect of presymptomatic start of cysteamine treatment

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RoMed Kliniken, Rosenheim

Newborn Screening

" Newborn screening (NBS) is a public health program of screening in infants shortly after birth **for conditions that are treatable, but not clinically evident in the newborn period.**

The goal is to identify infants at risk for these conditions early enough to confirm the diagnosis and provide intervention that will alter the clinical course of the disease and prevent or ameliorate the clinical manifestations".

Wikipedia

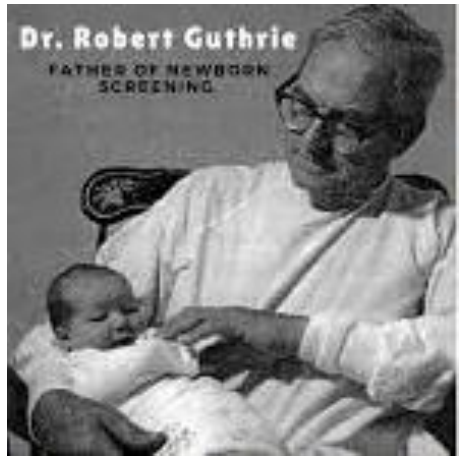
Newborn Screening- Germany

Tandem-Mass-Spectroscopy

Newborn screening
for 13 diseases

1960-USA — 1968 -Germany — 1970 — 2005 — 2017

Phenylketonuria



Galactosemia (classic)
Congenital Hypothyroidism
Congenital adrenal hyperplasia

Genetic Diagnostics

Cystic fibrosis

Guthrie R, Susi A:. Pediatrics 1963; 32: 338–42.
Chace DH, Acta Paediatr Suppl 1999; 88: 45–7
Naehrig S Dtsch Arztebl Int 2017;114(33–34):564–74.

Newborn Screening: Germany (14 target diseases)

CYSTINOSE STIFTUNG

Newborns: 770 000 / year

- Screenings: 680 000 (88 %)

  controls: 21 000 (2.95 %)

→ Detection of 700 newborns (0,1%)

- Costs

  newborn 30€

  year 22.000.000 €/y

→ Cost of undiagnosed patients

e.g. Hypothyroidism 1:3000-4000;

200 newborns/year/Germany

~ 2. 500. 000 € lifetime cost

Newborn Screening Programs

country- specific
dependent on

- healthcare system
- socio-economic
- cultural
- ethical
- legal requirements

major differences

target diseases
processing time (blood sampling)
organization
analytics

Therrell B.L. Current status of newborn screening worldwide:
Semin Perininatol 2015;39
Cornel M, Newborn Screening in Europe Expert opinion, 02,2022;
https://isnsneoscreening.org

Introduction of a new target disease

Detailed analysis of the entire screening process

robust scientific background
ethics
analytics
information technology
logistics
costs

potential benefit ?
reduction in
morbidity and mortality

potential harm ?
overdiagnosis,
false positives, false negatives

Alonso-Coello P., et al 2016
A systematic and transparent approach
to making well informed healthcare choices. BMJ

Wilson & Jungner's principles of screening (1968)

1. The condition sought should be an important health problem.
2. There should be an accepted treatment for patients with recognized disease.
3. Facilities for diagnosis and treatment should be available.
4. The disease cannot be diagnosed clinically with certainty in the neonatal period.
5. There should be a suitable test or examination.
6. The test should be acceptable to the population.
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.
8. There should be an agreed policy on whom to treat as patients.
9. The cost of case finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
10. Case finding should be a continuing process and not a "once and for all" project.

Wilson J.M.G.,Jungner G.,1968
World health Organization
Principles and Practice of Screening for Disease

Newborn Screening

Cystinosis

- effective therapy
- postnatal: no clinical signs

time at diagnosis (mean age, 14 months)

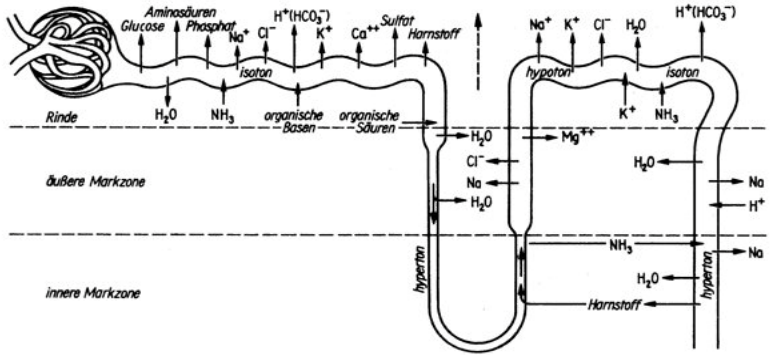
→ substantial renal damage

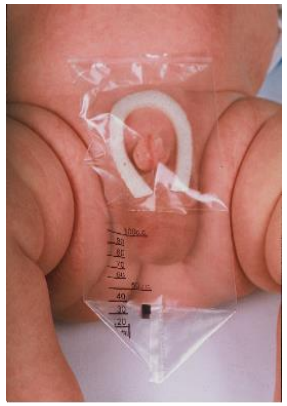
diagnosis: elevated cystine levels in white blood cells

→ unsuitable for screening

→ (molecular based) newborn screening ?

Newborn Screening for Cystinosis: Project 1 (n ~ 3000 newborns)





[Debré-Toni-Fanconi-Syndrom](#)

→ aminoaciduria
→ glycosuria

Methode: Urine dip-stick test

Advantage : cheap
additional identification of patients with glucosuria: neonatal diabetes, tyrosinemia

Disadvantage:

→ no screening infrastructure
→ patients without glycosuria[1]
→ no specific screening method

[1]Kanthila,J, Dsa S,Bhat K (2015) J Clin Diagn Res, 9(3) SD05–SD06.

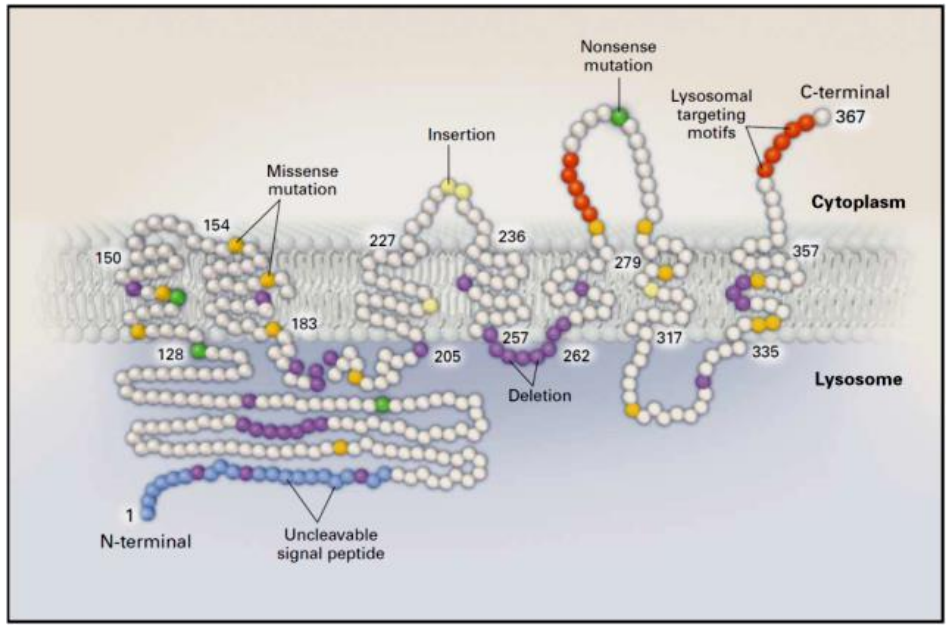
Newborn Screening for Cystinosis: Project 2

Routine German newborn screening
voluntary national health program (1968)

established screening structures
- covered by health insurances
- written informed consent
- dried blood spot cards
- 36 - 72 hours after birth
- analyzed at certified screening laboratories
- routine NBS- processed in 24 hours
- immediate information of positive results

Bekanntmachung eines Beschlusses des Gemeinsamen Bundesausschusses über eine Änderung der Richtlinien des Bundesausschusses der Ärzte und Krankenkassen über die Früherkennung von Krankheiten bei Kindern bis zur Vollendung des 6. Lebensjahres (Kinder-Richtlinien) zur Einführung des erweiterten Neugeborenen-Screenings.
BAnz. Nr. 60 vom 31.03.2005, 4833–8. Fassung vom: 18.06.2015 BAnz AT 18.08.2016 B1,Letzte Änderung: 19.10.2017 BAnz AT 15.03.2018 B2, In Kraft getreten am: 16.03.2018

Cystinosis: Cystinosin (CTNS) gene mutations > 100 identified



Gahl et al, NEJM 2002

Germany[1]

57.2kb Deletion

357delGATC

(HGVS nomenclature: c.18_21delGACT, p.T7Ffs * 7)

1261insG

(HGVS nomenclature: c.926dupG, p.S310Qfs * 55)

Familie	DNA Nr Alt	Name	Allel 1	Allel 2
60	60/98	LM	57-kb Deletion	400DelGGT
62	62/201	BN	57-kb Deletion	57-kb Deletion
65	65/201	WJ	57-kb Deletion	1033InsCG
76	76/201	MC	57-kb Deletion	632InsA
76	76/202	MJ	57-kb Deletion	632InsA
82	82/202	JO	57-kb Deletion	57-kb Deletion
85	85/201	SH	1261InsG	
91	91/301	SJ	57-kb Deletion	57-kb Deletion
96	96/204	AM	357delGACT	357delGACT
109	109/202	SM	57-kb Deletion	
114	114/202	KR	57-kb Deletion	357delGACT
114	114/201	KA	57-kb Deletion	357delGACT
122	122/201	BB	57-kb Deletion	57-kb Deletion
126	126/202	KM	57-kb Deletion	1262G>T
127	127/201	SC	57-kb Deletion	57-kb Deletion
131	131/201	WF	1261InsG	1261InsG
133	133/201	BJ	57-kb Deletion	57-kb Deletion
133	133/202	BJ(2)	57-kb Deletion	57-kb Deletion
176	176/204	EB	357delGACT	357delGACT
195	195/301	HP	57-kb Deletion	57-kb Deletion
197	197/201	WE	57-kb Deletion	
200	200/201	MA	57-kb Deletion	57-kb Deletion
200	200/202	MJ	57-kb Deletion	57-kb Deletion
201	201/201	GM	57-kb Deletion	57-kb Deletion
201	201/202	GM(2)	57-kb Deletion	57-kb Deletion
201	201/203	GJ	57-kb Deletion	57-kb Deletion
206	206/201	FF	1261InsG	1261InsG
209	209/302	KS	57-kb Deletion	57-kb Deletion
211	211/201	GC	357delGACT	357delGACT

1Kiehntopf M, et al..
Hum Mutat 2002;20(3):237–237.

Two-Tier Strategy

1. PCR reactions

57.2kb deletion
c.18_21delGACT, p.T7Ffs * 7
c.926dupG, p.S310Qfs * 55

→ ~ 75% of homozygous or heterozygous patients

2. Heterozygous probes

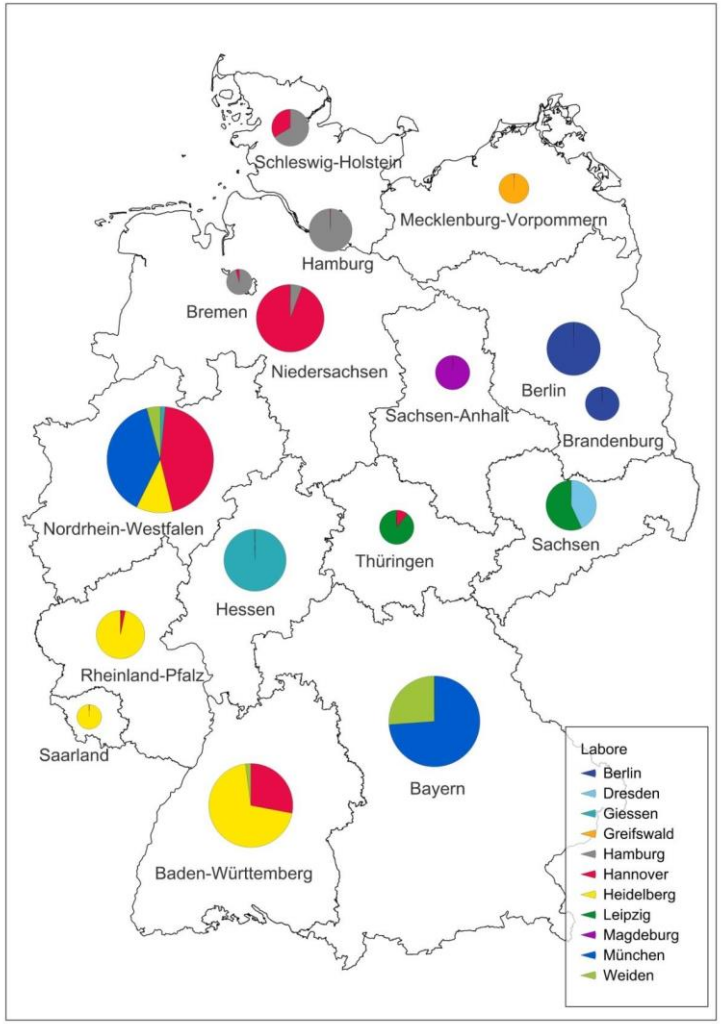
Next- generation Sequencing (NGS) for 101 mutations

→ ~ 98.5% of patients with cystinosis

estimated heterozygosity of *CTNS* mutations
prevalence of disease: 1 : 100.000 and 1 : 200.000

→ in 100.000 probes 500 heterozygous probes are expected

Screening for cystinosis and SMA - 15.01.2018



Screening labratories

Becker and colleagues, Munich

~ 110 000 screening probes/year

Janzen, Hannover

~ 250 000 screening probes/year

Ethics Committee of the Bayerische Landesärztekammer
(BLAEK, Ethic permit No.16125)
Ethics Committee of Hannover Medical School (No. 7772_BO_K_2018)

additional written informed consent
integrated in routine NBS

CYSTINOSIS RESEARCH NETWORK

gefördert durch DLH

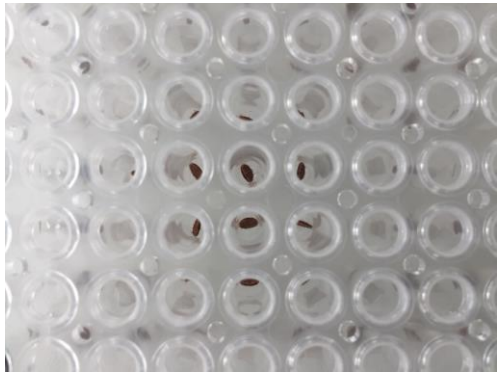
CYSTINOSE STIFTUNG

25 Jahre Sternstunden
WIR HELFEN KINDERN

CYSTINOSE STIFTUNG

1. DNA-extraction

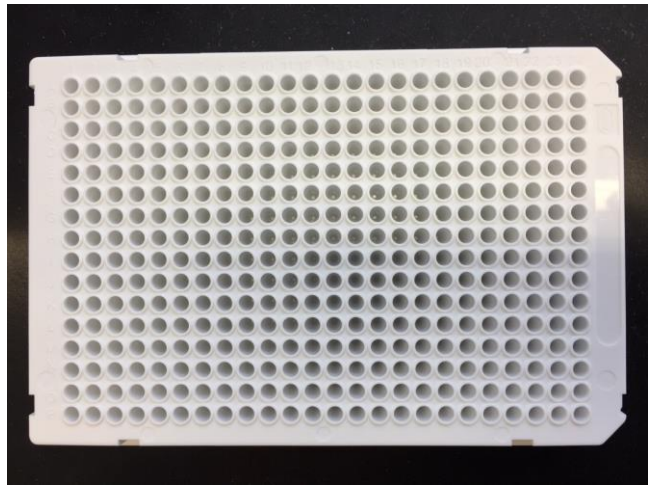
3.2 mm punches dried blood spot cards
96 probes/plate

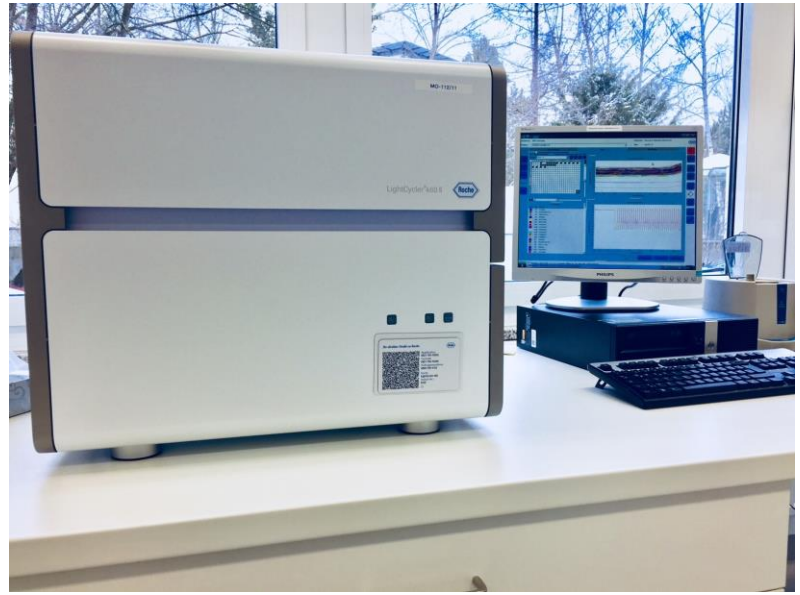


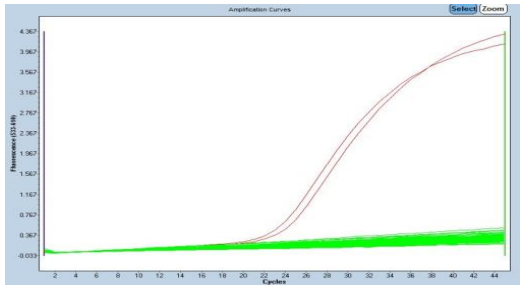
2. PCR-reactions

342 probes and controls- 4x96 plates with DNA
Simultaneous PCR-reactions for 4 parameters (multiplex analysis)

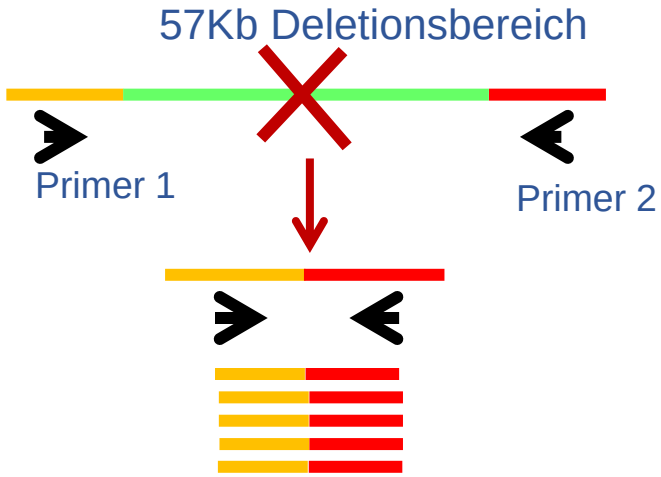
a. 57 kb deletion
   differentiation heterozygous/homozygous
b. 357DelGACT-Mutation (c.18_21delGACT)
c. 1261InsG-Mutation (c.926dupG)
d. SMA



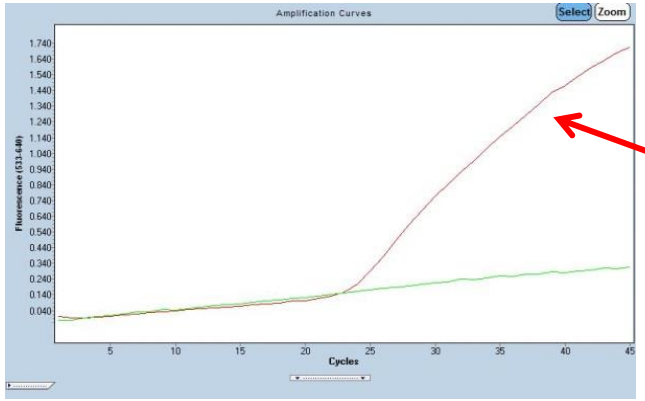




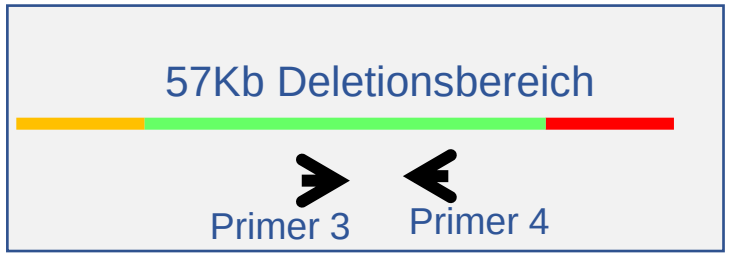
both alleles are intact
no PCR reaction



Next-generation sequencing for 101 known mutations



57-kb deletion in one allele
heterozygous patient



Cystinosis screening results: n= 299 631 (15.01.2018 – 15.02.2020)

Heterozygous probes:	57 kb	n= 655
	c.18_21del GACT	n= 85
	c.926dupG	n= 65

Homozygous deletion:	n=1	57 kb deletion (leucocyte cystine level: 2.8 nmol cystine/mg protein) treatment started at day 18 no Fanconi syndrome at age 3,5 years
	n=1	57 kb deletion (leucocyte cystine level: 2.9 nmol cystine/mg protein) mother refused initial screening, diagnosis at 8 months of age
Compound heterozygous deletion	n=1	57 kb deletion, promotor variant c.-512G>C (leucocyte cystine level <0.2 nmol cystine/mg protein) ACMG –classification – none pathogenic mutation

Newborn screening for Cystinosis and SMA

- no false positive
- until now – no false negative
- adherence of NBS 88% in Bavaria
- implementation of SMA in routine NBS in Germany (2021)

Bekanntmachung eines Beschlusses des Gemeinsamen Bundesausschusses über eine Änderung der Kinder-Richtlinie: Neugeborenen-Screening auf 5q-assoziierte spinale Muskelatrophie vom: 17. Dezember 2020

Project 3

Newborn screening for Cystinosis and Hyperoxaluria (31.03.2022)

- In cooperation with the German Society of Pediatric Nephrology (GPN)
- Prospective data collection of patients identified in screening and of patients who have been clinically diagnosed.

Project 3 Newborn screening for Cystinosis and Hyperoxaluria (31.03.2022)

Screening-Labor Hannover, Hannover Germany

Cystinosis

57.2kb Deletion
c.18_21delGACT, p.T7Ffs * 7

→ heterozygous probes: NGS for 174 mutations (same blood spot card)
(Prof. C. Bergmann, Labor Limbach, Mainz, Germany)

Hyperoxalurie

PH 1, c.508G>A (infantile hyperoxaluria, most common mutation)
PH 3, c.700+5G>T (most common European mutation)

→ heterozygous probes: urine diagnostic, genetic diagnostic
(Prof. B. Hoppe, Bonn, Germany, Dr. B. Beck, Cologne, Germany)

Conclusion: Newborn screening for Cystinosis

- molecular-based newborn screening for Cystinosis is feasible
- heterozygous probes can be further evaluated with NGS for specific mutations from the same dried blood spot card
- future perspective:
  implementation of Cystinosis (57-kb) in German routine NBS

  → sibling data (K. Veys)
  → cystinosis guideline

  → longitudinal tubular data
  → longitudinal glomerular data

Tubular Function – early cysteamine treatment

n = 6
160 visits

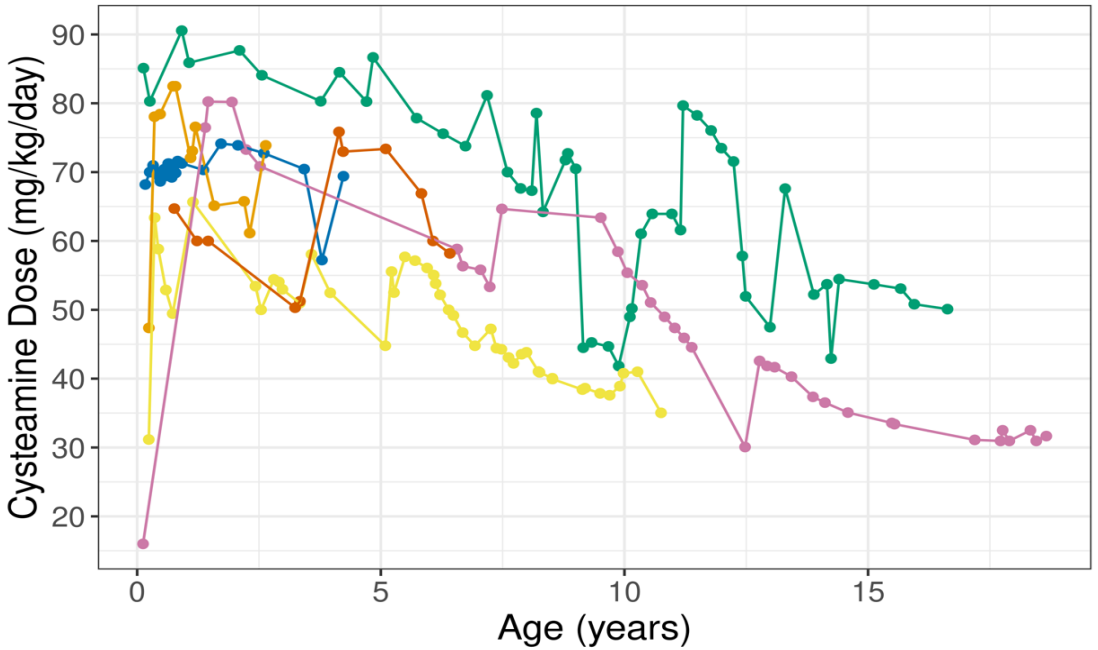
Patient #	105	88	**5**	**74**	8[a]	99
Age at start of cysteamine (mo)	**1.6**	**0.9**	**0.4**	**0.2**	**0.4**	**0.7**
Age at last visit (y-mo)	**2.7**	**4.2**	**16.7**	**10.9**	**18.7**	**6.5**

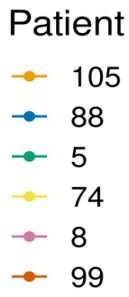
Height (Percentile)	90	27	**52**	**58**	(173 cm)	49
Weight (Percentile)	86	14	**14**	**88**		59
Weight (kg)	15.7	15.1	**49.9**	**47.1**	62.7	23.2

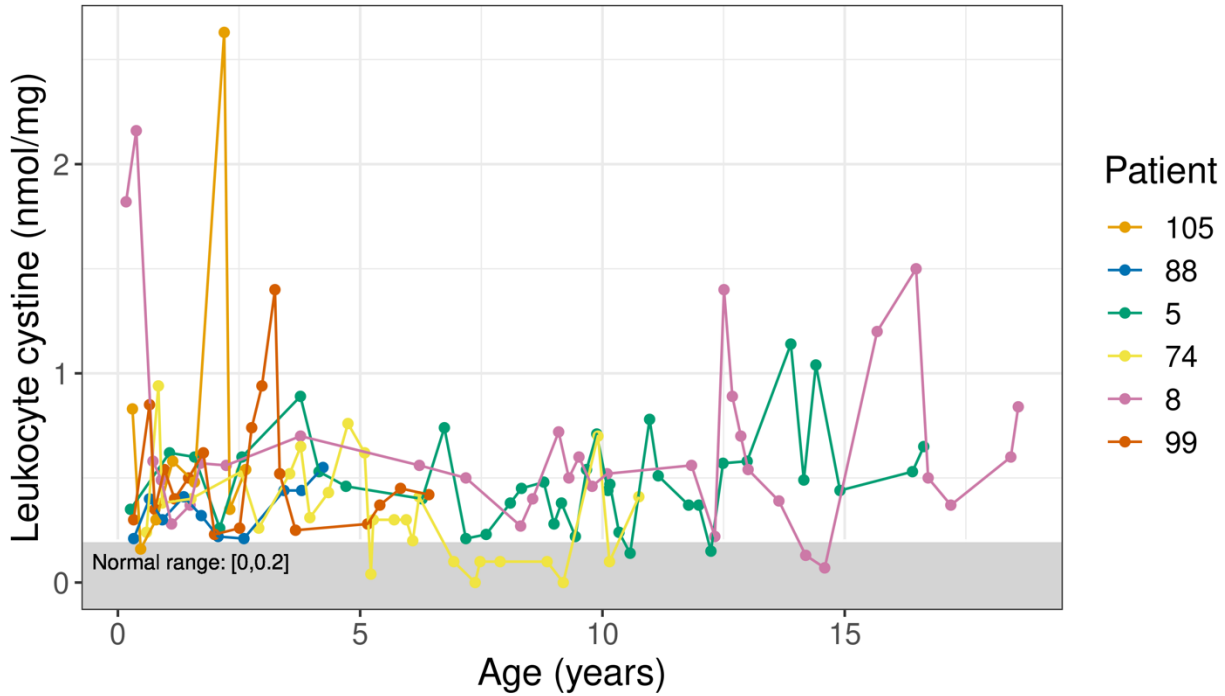
[a] Patient 8 received growth hormone and thyroid hormone.

Beneficial Effects of Starting Oral Cysteamine Treatment in the First 2 Months of Life on Tubular Kidney Function in Infantile Nephropathic Cystinosis
K. Hohenfellner, ,C. Nießl, A-L Boulesteix, D. Haffner[4], J. Oh, K. Palm, L. Pape, K Schlingmann, S. Wygoda, W. Gahl, Molecular Genetics and Metabolism, 2022

Cysteamine Dose - Leukocyte cystine (nmol/mg)

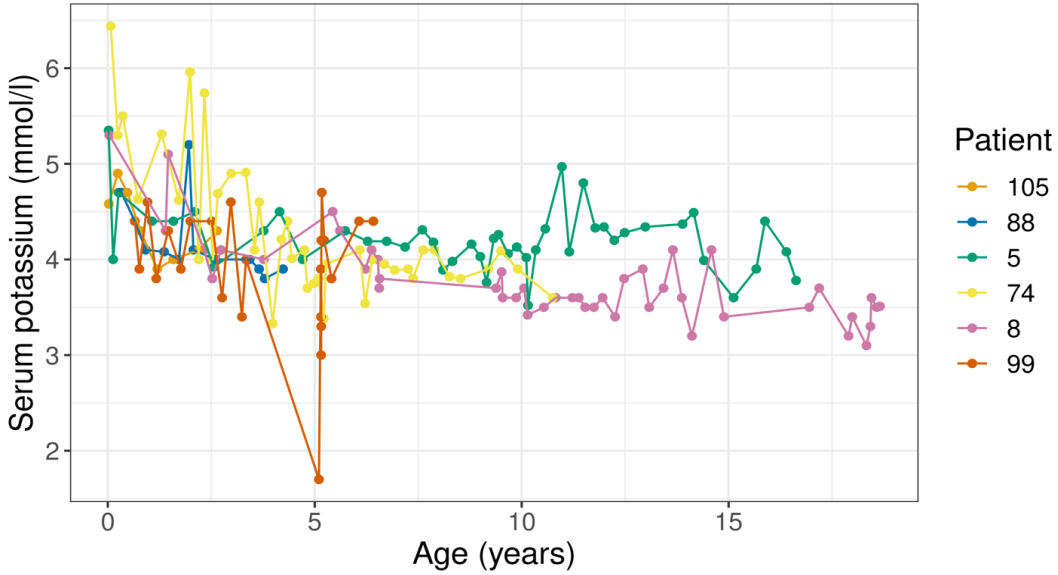






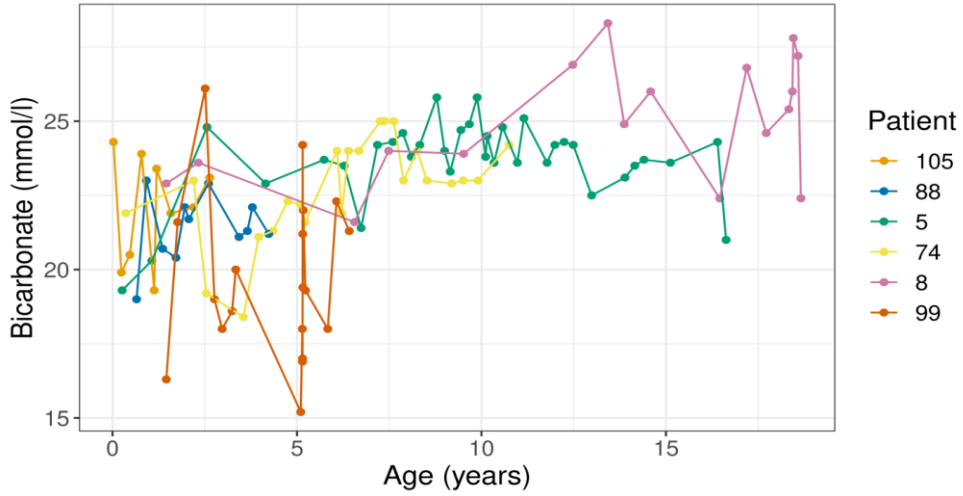
Electrolytes

Patient #	**105**	**88**	**5**	**74**	**8**	**99**
Age (y-mo)	2-7	4-2	16-7	10-9	18-7	6-5
Oral replacement						
Potassium	No	No	No	No	Yes	Yes
Bicarbonate	No	No	No	No	Yes	Yes
Citrate	No	No	No	No	No	Yes
Phosphate	No	No	No	No	Yes	Yes
Calcium	No	No	No	No	No	Yes



Serum values

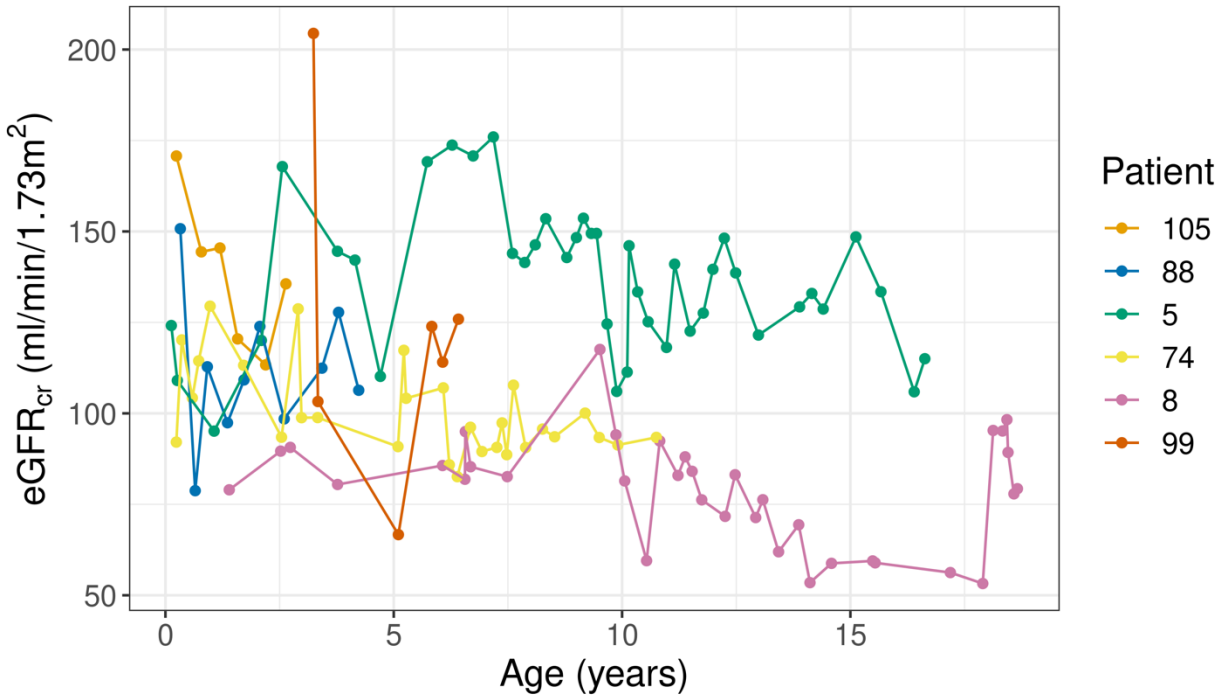
Potassium (mmol/L)	4.3	3.9	3.8	3.6	3.5	4.4
Bicarbonate (mmol/L)	23.1	21.2	21.0	24.2	22.4	21.3
Phosphate (mmol/L)	1.64	1.52	1.23	1.15	0.74[a]	1.58
Calcium (mmol/L)	2.42	2.38	2.24	2.34	2.46	2.26



Urine values

Spot urine values							
Creatinine (g/l)	0.61	0.54	0.96	0.7	0.26	0.29	Variable
Albumin (mg/l)	12.5	5.9	95.8	8.1	58.3	111.6	< 20
mg/g Creatinine	21	11	100	12	224	385	< 20
IgG (mg/l)	4.3	<4.0	30.8	11.4	12.7	18.1	< 14
mg/g Creatinine	7.0	<7.4	32.1	16.3	48.8	62.4	<10
α_1-Microglobulin[c] (mg/l)	27.5	7.5	153	23	44	107	< 20
mg/g Creatinine	45	14	158	33	169	367	< 10
α_2-Macroglobulin (mg/l)	<4.0	<4.0	<4.0	<2.41	<4.0	<4.0	< 20
mg/g Creatinine	< 6.6	<7.4	<4.2	<2.4	<15.4	<13.8	< 10
Urine values							
Volume (mL/24h)	600	700	2000	1600	7400	1300	
TRP (%)	84	91	88	84	45	72	82-90
FSI (µmole/kg/day)	318	176	425	208	1236	560	49-139 [b]
TmP/GFR (mg/dl)	4.6	1.8	2.7	3.9	0.9	3.5	

eGFR



Patient #	105	88	5	74	8	99
Age at start of cysteamine (mo)	1.6	0.9	0.4	0.2	0.4	0.7
Age at last visit (y-mo)	2-7	4-2	16-7	10-9	18-7	6-5
$eGFR_{cr}$ at last visit (mL/min/1.73 m²)	136	106	115	93	79	126

Organization

Cystinose Foundation , Munich, Germany

Laboratory and screening partners

Screening-Labor Hannover
Dr. med. Dr. rer. nat. Nils Janzen, Hannover, Germany

Labor Becker & Kollegen MVZ GbR, Munich, Germany
Dr. med. Marc Becker
Dr. rer. nat. Siegfried Burggraf

Human genetics partner

Prof. Dr. med. Carsten Bergmann, Labor Limbach, Mainz, Germany
Priv. Doz. Dr. Bodo Beck, Institut fuer Humangenetik Koeln, Germany

Hyperoxaluria partner

Prof. Bernd. Hoppe, Hyperoxaluria Center Germany, Bonn, Germany

SMA partner

Prof. Dr. med. Wolfgang Müller-Felber, Munich, Germany

Thank you for your attention !