Controversies Conference on Nephropathic Cystinosis: Diagnostics and Biochemical Follow-up

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Disclosure of Interests

Bruce A. Barshop, MD, PhD

- Genzyme-Sanofi: Research Grant
- Pfizer: Honorarium/ Sponsored Education
- BioMarin: Sponsored Education

No other conflicts of interest to report.

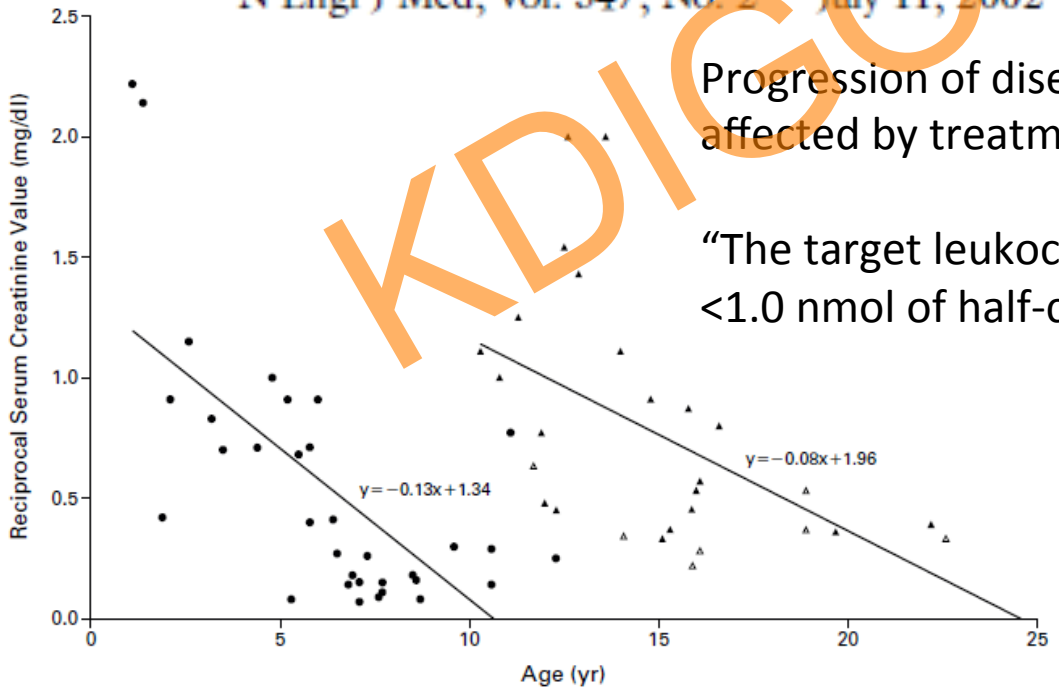
why monitor?

(Not controversial.)

CYSTINOSIS

WILLIAM A. GAHL, M.D., PH.D., JESS G. THOENE, M.D., AND JERRY A. SCHNEIDER, M.D.

N Engl J Med, Vol. 347, No. 2 · July 11, 2002



Progression of disease dramatically affected by treatment with cysteamine.

"The target leukocyte cystine content is <1.0 nmol of half-cystine per mg protein."

target range

Predicted reciprocal serum creatinine at age 10 years as a measure of renal function in children with nephropathic cystinosis treated with oral cysteamine

William A. Gahl[1], Jerry A. Schneider[2], Joseph D. Schulman[3], Jess G. Thoene[4], and George F. Reed[5]

Pediatr Nephrol (1990) 4: 129–135

Table 3. Stratification according to leukocyte cystine levels

	Leukocyte cystine depletion groups[a]			
	1	2	3	4
n	19	18	16	18
Initial age (years)	3.27 ± 0.52[b]	2.98 ± 0.37	2.90 ± 0.55	4.20 ± 0.43
Initial creatinine (mg/dl)	0.87 ± 0.07	0.91 ± 0.09	0.80 ± 0.10	1.29 ± 0.10
Creatinine clearance (ml/min per 1.73 m^2)				
– initial	56.6 ± 4.4	57.8 ± 6.0	62.42+ ± 5.5	43.0 ± 5.1
– final	64.6 ± 6.6	59.2 ± 6.4	63.0 ± 7.0	37.9 ± 6.9
– (final-initial)	8.0 ± 5.8	1.4 ± 4.2	0.5 ± 8.1	–5.0 ± 3.4
*P**		0.374	0.450	0.066
PRC_{10}	0.96 ± 0.11	0.65 ± 0.12	0.68 ± 0.20	0.35 ± 0.18
*P**		0.064	0.203	0.006

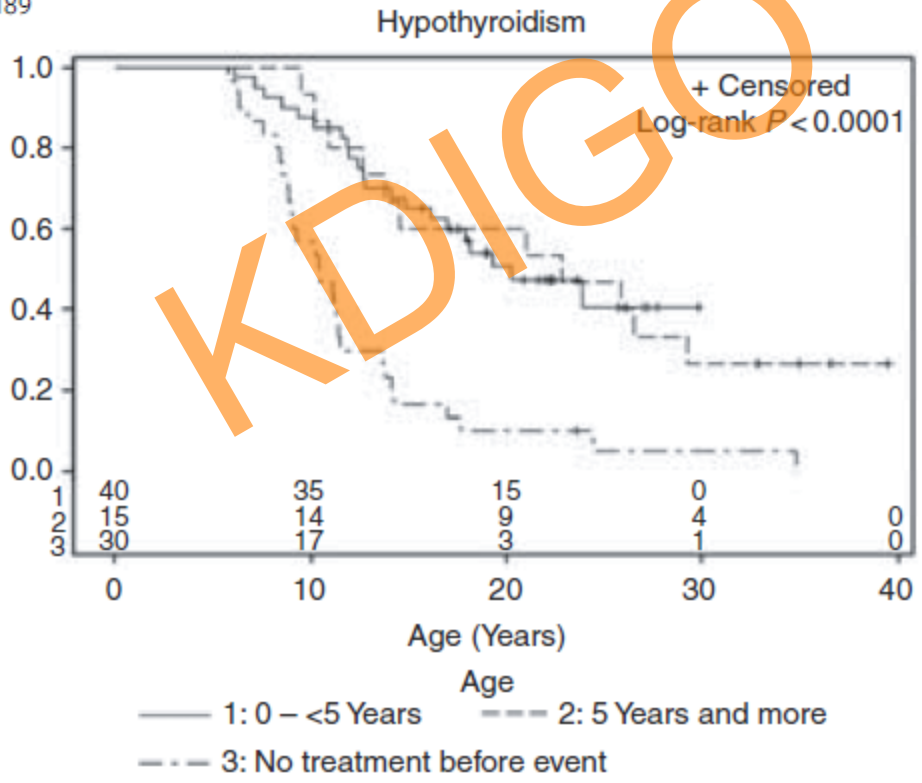
[a] Groups 1–3: Two or more leukocyte cystine levels obtained per year
Group 1: Median level <1 nmol $^1/_2$ cystine/mg protein
Group 2: Median level between 1 and 2 nmol $^1/_2$ cystine/mg protein
Group 3: Median level over 2 nmol $^1/_2$ cystine/mg protein
Group 4: Fewer than two levels obtained per year or could not tolerate full recommended dose of cysteamine
[b] SEM
* Two sided *P* value compared with group 1, using Student's *t*-test

earliest treatment/ earliest measurement

Cysteamine therapy delays the progression of nephropathic cystinosis in late adolescents and adults

Albane Brodin-Sartorius[1,2], Marie-Josèphe Tête[2,3], Patrick Niaudet[2,3], Corinne Antignac[2,4,5], Geneviève Guest[2,3], Chris Ottolenghi[2,6], Marina Charbit[2,3], Dominique Moyse[7], Christophe Legendre[2,8], Philippe Lesavre[1,2], Pierre Cochat[9,10], Aude Servais[1,2]

Kidney International (2012) **81**, 179–189



pre-symptomatic diagnosis/ screening

- **Screening = Population-based**
  - **e.g. newborn screening, Pap smear, mammograms**
- **Carrier testing**
  - **e.g. Dor Yeshorim for Tay Sachs carriers in orthodox Jewish communities**
- **Presymptomatic testing**
  - **e.g. pregnancy in progress in family at risk for cystinosis**
- **Preimplantation diagnosis**
  - **e.g. planned pregnancy in family at risk**

pre-symptomatic diagnosis/ screening

- **How soon to test post-natal?**
  - **No increased storage at birth, or at least non-diagnostic. Customary to wait… how long?**
- **Feasible *in utero*?**
  - **Biochemical: CVS … but risk to fetus unwarranted unless info used to inform decision to terminate**
  - **Genetic: CVS or amino – or NIPT (non-invasive prenatal testing, cell-free DNA in maternal circulation) …with same proviso**
  - **Genetic: Preimplantation**

Prenatal diagnosis of cystinosis by quantitative measurement of cystine in chorionic villi and cultured cells

Marie Jackson[1] and Elisabeth Young[1,2]*

Prenat Diagn 2005; 25: 1045–1047.

Table 1—Number of pregnancies at risk for cystinosis monitored

Sample	Method	Total	Not affected	Affected
CAC	[^{35}S]-cystine uptake	72	57	15
CV + CAC	[^{35}S]-cystine uptake	4	4	0
CV	[^{35}S]-cystine uptake	54	44	10
CCV	[^{35}S]-cystine uptake	3	3	0
CV	Quantitative assay & [^{35}S]-cystine uptake	12	9	3
CV	Quantitative assay	13	9	4
CCV	Quantitative assay	1	1	0
CAC	Quantitative assay	1	1	0
TOTAL		160	128	32

CAC, cultured amniotic cells; CV, chorionic villi; CCV, cultured chorionic villi cells.

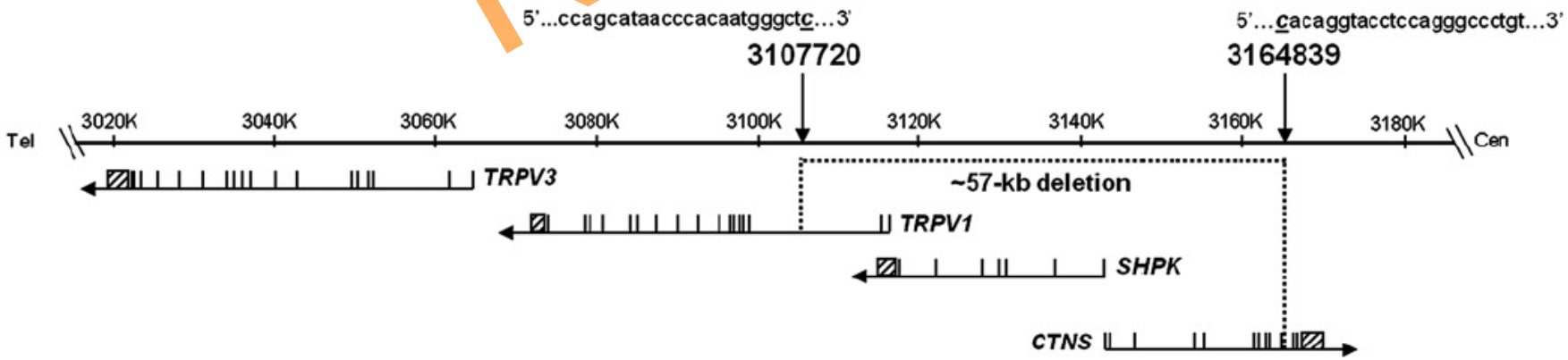
pre-symptomatic diagnosis/ screening

- Common 57b kb deletion found to also delete CARKL (SHPK) (Touchman et al., 2000; Phornphutkul et al., 2001) and extend into TRPV1 (Freed et al., 2001).

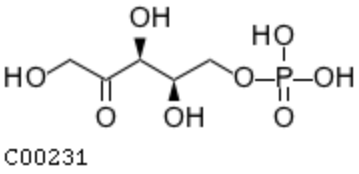
Katy A Freed,[1] John Blangero,[1] Tom Howard,[2] Matthew P Johnson,[1] Joanne E Curran,[1] Yvonne R Garcia,[1] Hao-Chang Lan,[1] Hanna E Abboud,[3] Eric K Moses[1]

The 57 kb deletion in cystinosis patients extends into *TRPV1* causing dysregulation of transcription in peripheral blood mononuclear cells

J Med Genet 2011;**48**:563–566.

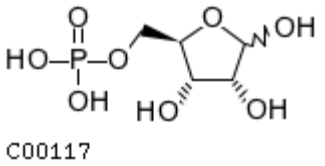


sedoheptulokinase

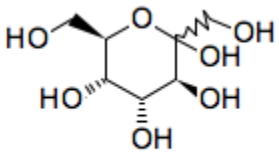


D-Xylulose 5-phosphate

TKT

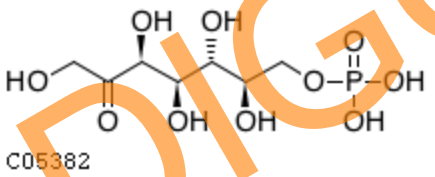


D-Ribose 5-phosphate

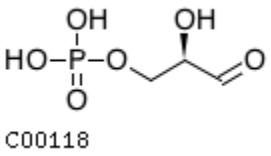


D-Sedoheptulose, Volemulose

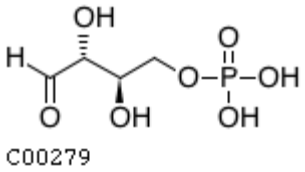
SHPK, CARKL



Sedoheptulose 7-phosphate

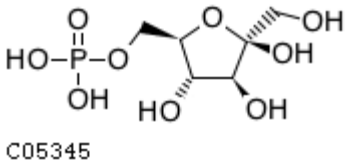


Glyceraldehyde 3-phosphate



D-Erythrose 4-phosphate

TALDO1



D-Fructose 6-phosphate

sedoheptulokinase deficiency

Characterization of mammalian sedoheptulokinase and mechanism of formation of erythritol in sedoheptulokinase deficiency

Tamas Kardon[a], Vincent Stroobant[b], Maria Veiga-da-Cunha[a], Emile Van Schaftingen[a,*]

FEBS Letters 582 (2008) 3330–3334

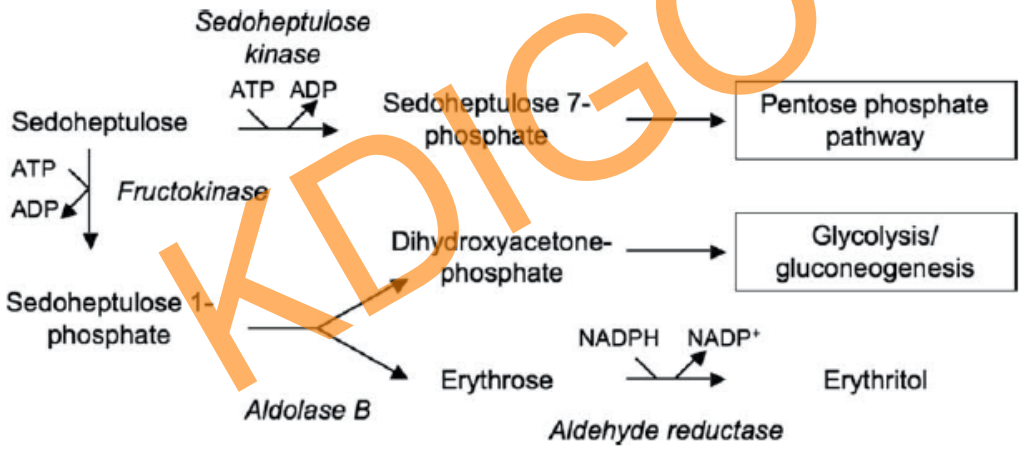


Fig. 3. Metabolism of sedoheptulose and its alteration in sedoheptulokinase deficient patients. Sedoheptulokinase normally converts sedoheptulose to sedoheptulose 7-phosphate, which is metabolized by enzymes of the pentose phosphate pathway. Based on the properties of fructokinase and aldolase B, we propose that, in the absence of sedoheptulokinase, sedoheptulose would be phosphorylated by fructokinase to sedoheptulose 1-phosphate, which would then be cleaved by aldolase B to dihydroxyacetone-phosphate and erythrose. The latter would be reduced to erythritol by aldehyde reductase. Both sedoheptulose and erythritol are excreted in urine.

pre-symptomatic diagnosis/ screening

- **Feasible screening? Biomarker for 57 kb deletion.**

Elevated concentrations of sedoheptulose in bloodspots of patients with cystinosis caused by the 57-kb deletion: Implications for diagnostics and neonatal screening

M.M.C. Wamelink [a,*], E.A. Struys [a], E.E.W. Jansen [a], H.J. Blom [a], T. Vilboux [b], W.A. Gahl [b], M. Kömhoff [c], C. Jakobs [a], E.N. Levtchenko [d]

Molecular Genetics and Metabolism 102 (2011) 339–342

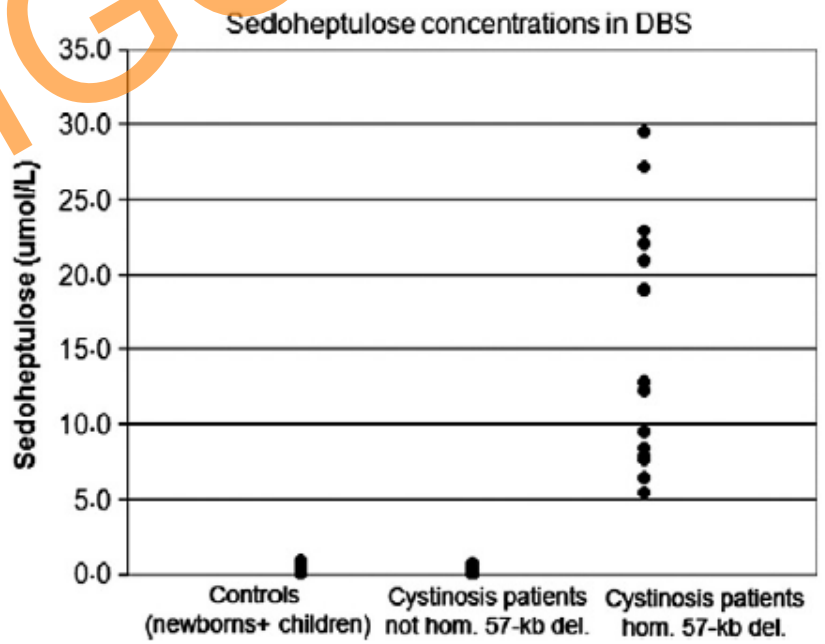


Fig. 2. Scatter plot of sedoheptulose concentrations in DBS measured via LC–MS/MS.

ctns mutations

Missense/nonsense	50
Splicing	15
Regulatory	2
Small deletions	24
Small insertions	10
Small indels	4
Gross deletions	12
Gross insertions	0
Complex	0
Repeats	0

The Human Gene Mutation Database
at the Institute of Medical Genetics in Cardiff
21-Nov-2014

Mutational Spectrum of the CTNS Gene in Egyptian Patients with Nephropathic Cystinosis

Neveen A. Soliman • Mohamed A. Elmonem • Lambertus van den Heuvel • Rehab H. Abdel Hamid • Mohamed Gamal • Inge Bongaers • Sandrine Marie • Elena Levtchenko

JIMD Reports 2014

Fig. 1 Worldwide geographical distribution of 57-kb deletion and previously reported *CTNS* mutations detected in the Egyptian population. *Oval*: geographical distribution of 57-kb deletion. *Circle*: geographical distribution of the Middle Eastern mutation c.681G>A

common mutations/ uncommon mutations

- Owen, et al. **Common mutation causes cystinosis in the majority of black South African patients. Pediatr Nephrol** 2014
  - 19/20 pts: CTNS c.971-12G > A p.D324AfsX44, intron 11 out-of-frame 10-bp ins. 16/19 homozygous.
- Shahkarami, et al. **The first molecular genetics analysis of individuals suffering from nephropatic cystinosis in the Southwestern Iran.** Nefrologia 2013;33:308-15.
  - 0/25 pts had the 57 kb deletion (het or homo). 1/25 hom novel mut, c.153-155insCT, 1/25 hom and 1/25 cpd het with c.923G>A. Also three known muts: c.18-21delGACT, c.1017G>A, and c.681G>A in 11/25. No mut detected in 11/25 pts.
- Topaloglu et al. **Genetic basis of cystinosis in Turkish patients: a single-center experience.** Pediatr Nephrol. 2012 27(1):115-21.
  - 0/12 patients had the 57-kb deletion. 4 known variations (c.140+1 G>T, c.1015 G>A (p.G339R) , c. 18_21del GACT (p.T7FX7), c.681 G>A (p.E227E)), 5 new variants: a 10-kb deletion (c. 62-1083_551del10217bp), 3 missense variants (c.518A>G (p.Y173C), c.451A>G (p.R151G), c.470 G>A (p.G157D)), and a nucleotide substitution in a potential branch point site of intron 4 (c.141-22a>g).
- Soliman, et al. **Mutational Spectrum of the CTNS Gene in Egyptian Patients with Nephropathic Cystinosis.** JIMD Rep 2014
  - 0/15 patients had 57-kb deletion; 27 mutant alleles and 12 pathogenic mutations detected, incl. 6 novel mutations.
- Mason et al. **Mutational spectrum of the CTNS gene in Italy.** EJHG 2003 11, 503–508.
  - 57-kb deletion only in 17% of 84 chromosomes. Several splice site mutations.

pre-symptomatic diagnosis/ screening

- **Biomarker for 57 kb deletion: Ethnically targeted?**

Ethical implications and practical considerations of ethnically targeted screening for genetic disorders: the case of hemoglobinopathy screening

Cynthia F. Hinton*, Althea M. Grant and Scott D. Grosse

Ethnicity & Health

Vol. 16, Nos. 4–5, August–October 2011, 377–388

1. Categories of race/ethnicity are social constructs, therefore, observed or self-identified broad racial/ethnic categories are not necessarily reliable indicators of geographic ancestry or genetic risk.
2, Targeting based on ethnicity poses serious issues of logistics and equity for public health programs and clinical services.

pre-symptomatic diagnosis/ screening

America's Churning Races:
Race and Ethnic Response Changes between Census 2000 and the 2010 Census

www.census.gov

Table 4. Non-Hispanic black, American Indian, and/or white response stability and change

Race response in 2000 Census linked data	Race response in 2010 Census linked data					
	B	AIAN	W	B & AIAN	B & W	AIAN & W
black (B)	14,881,514	**22,793**	**112,882**	**71,382**	**130,788**	
AIAN	**16,307**	723,326	**158,178**	**4,948**		**99,910**
white (W)	**102,464**	**173,415**	122,765,113		**67,879**	**404,209**
B & AIAN	**50,000**	**3,713**		16,433		
B & W	**90,086**		**35,837**		249,359	
AIAN & W		**87,809**	**339,481**			134,523

pre-symptomatic diagnosis/ screening

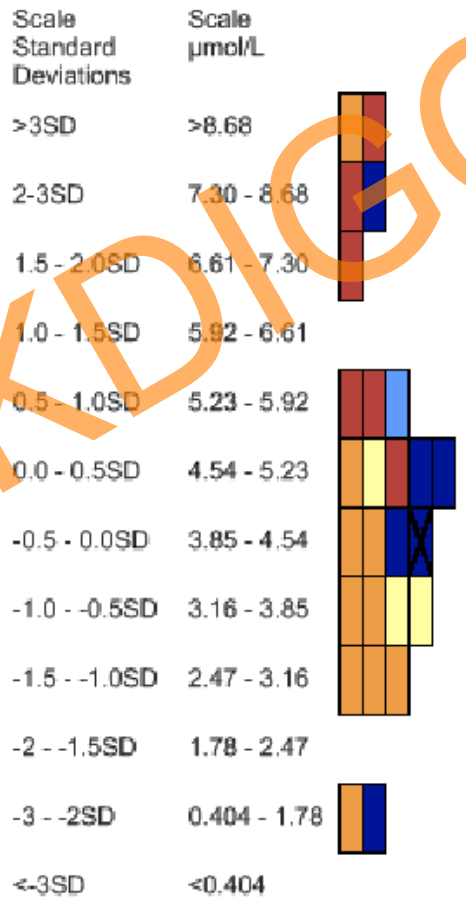
- Can apply a “universal”, “comprehensive” panel of mutations, but a) always changing, b) inherently discriminatory and disenfranchising for rare minorities.
- Better at present is to first use a (less specific) biomarker, and then use such a mutation panel to confirm findings, e.g. immunoreactive trypsinogen for cystic fibrosis.
- Presently no such biomarker exists for cystinosis….
- Things are changing quickly with regard to comprehensiveness of genetic/ genomic screening.

optimal technique for testing

- Amino acid analyzer- soon proved insufficiently sensitive.
- Cystine binding protein- very demanding, slow throughput limited supply.
- Tandem mass spectrometry (LC-MS/MS)- the methodology of choice presently.

ERNDIMQA - ANALYTE IN DETAIL

Cystine in White Blood Cells



optimal technique for testing

- Stabilization of -SH: inhibition of disulfide exchange
  - N-Ethylmaleimide: method of choice
  - Acidic storage (e.g. sulfosalicylic acid): may suffice and may be favored when cell isolation is done at a remote location.
- Stable isotope dilution: preferable with LC-MS/MS
- Quantification of protein may be the bigger issue in the analytical phase.

denominator effects

An unexpected problem in the clinical assessment of cystinosis

Kathy L. Powell · Craig B. Langman

Pediatr Nephrol (2012) 27:687–688

"Comparison of BCA and Lowry total protein assay values, using a bovine serum albumin standard, from 106 clinical samples from patients with cystinosis, revealed a significant and consistent difference in values from identical samples. The mean and standard deviation of the ratio between BCA and Lowry results over all samples was 0.65±0.07.
Discovery of the discrepancy in total protein values allowed values to be normalized and the study to proceed. We suspect that the discrepancies observed are based on variable assay sensitivity to different protein types, as has been noted…"

Though Lowry method and bicinchoninic acid (BCA) method agreed perfectly well on BSA standards and on ERNDIM protein unknowns (which were simply BSA), there were differential responses of the two methods when used on leukocyte lysates.

denominator effects/ optimal prep

Comparison of Cystine Determination in Mixed Leukocytes vs Polymorphonuclear Leukocytes for Diagnosis of Cystinosis and Monitoring of Cysteamine Therapy,

Clinical Chemistry 50, No. 9, 2004

Elena Levtchenko,[1] Adriana de Graaf-Hess,[2] Martijn Wilmer,[2] Lambertus van den Heuvel.[1,2] Leo Monnens.[1] and Henk Blom[2]*

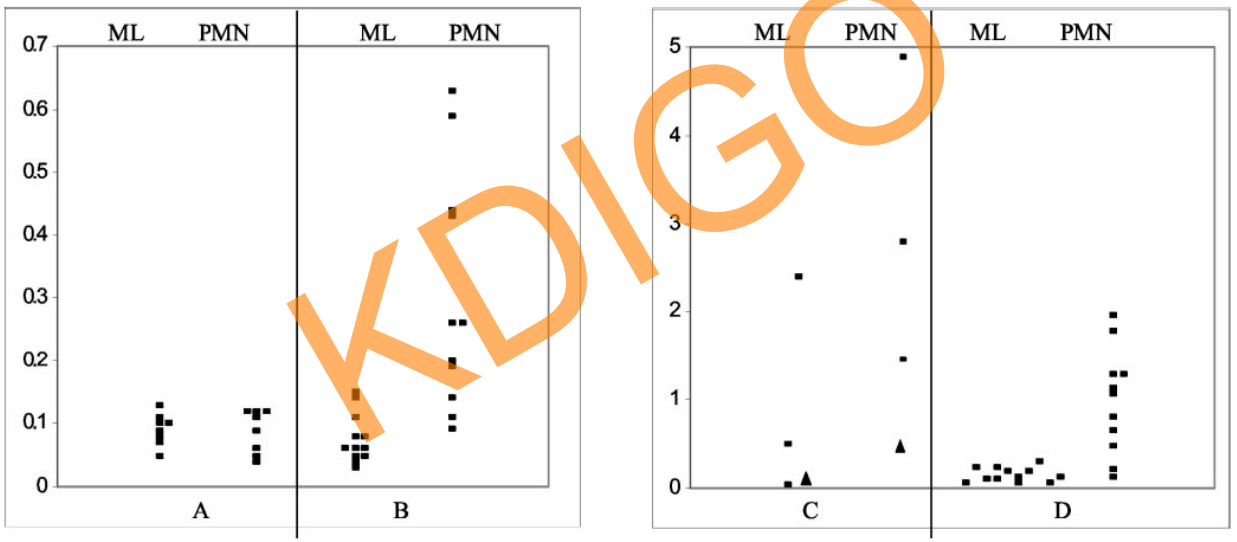


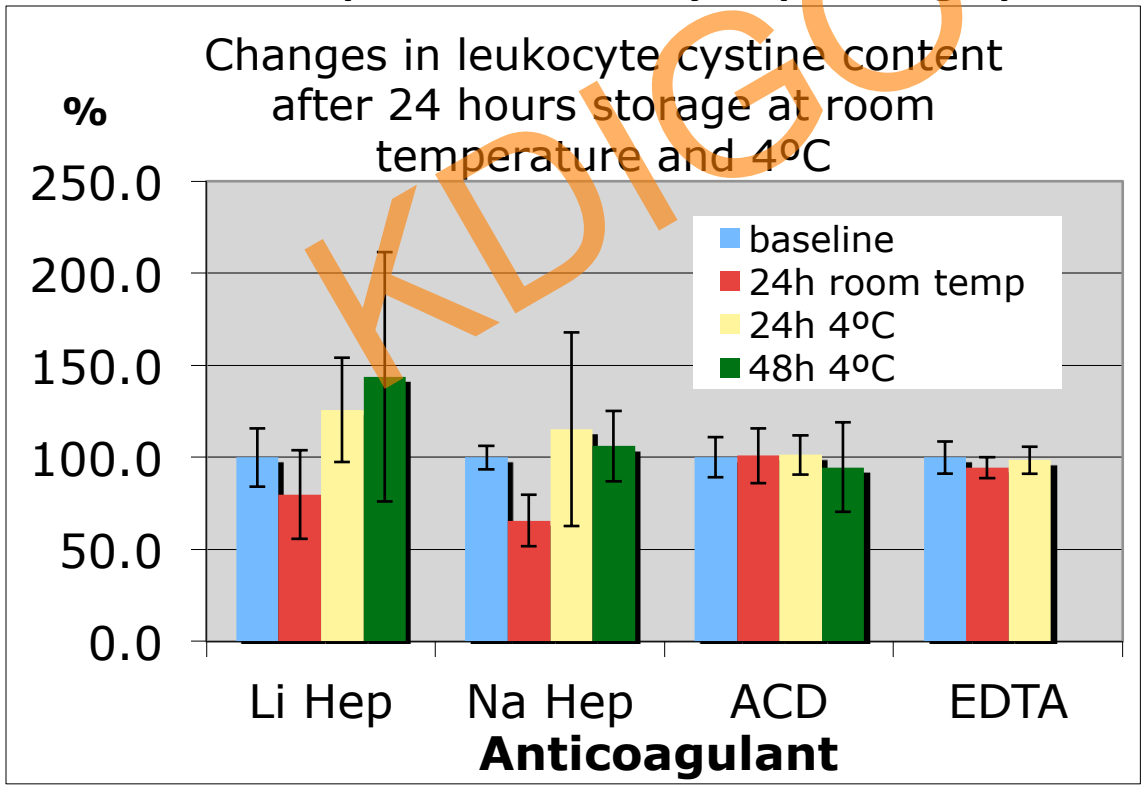
Fig. 1. Cystine (nmol/mg of protein) in ML preparations and PMN cells.

(*A*), healthy controls (n = 8); (*B*), obligate heterozygotes (n = 15); (*C*), patients at diagnosis (n = 4); (*D*), patients undergoing cysteamine therapy (n = 12). ▲, patient with late-onset cystinosis.

"Because we observed a clear difference between cystine content in ML preparations and PMN cells, we suggest that each laboratory produces its own reference values based on the upper cystine values found in heterozygotes."

optimal prep/storage

- **Problem is in pre-analytical phase, i.e. cell prep**
  - **Erratic results with prolonged storage of whole blood**
  - **Some anticoagulants are better than others- ACD**
    - **Differences in protein recovery depending upon anticoagulant**



Fidler *et al.*, 2011, unpublished

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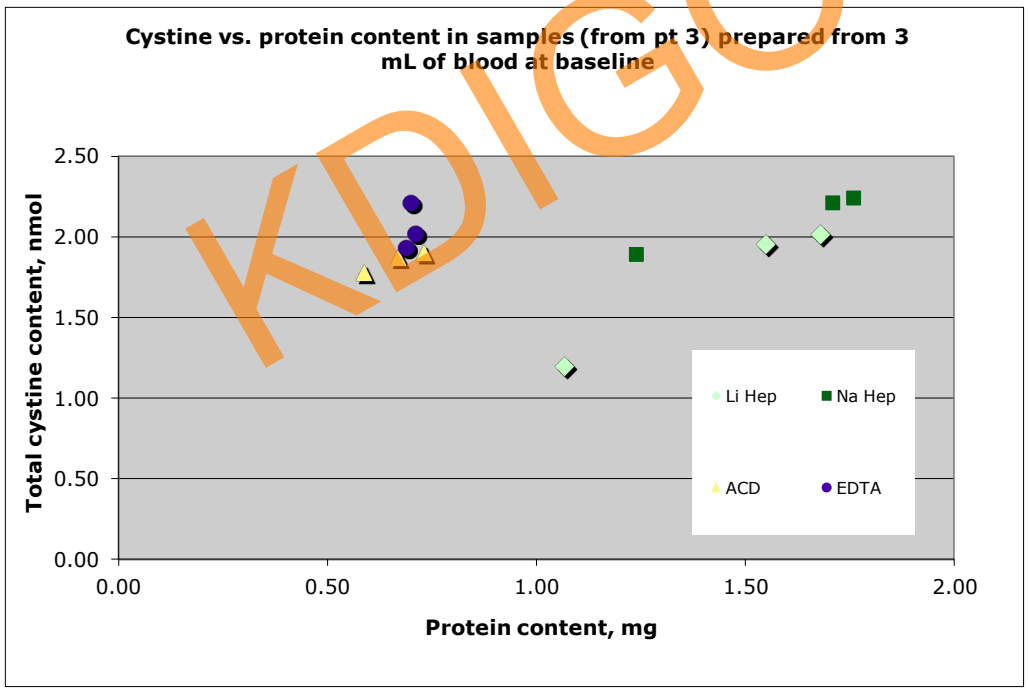

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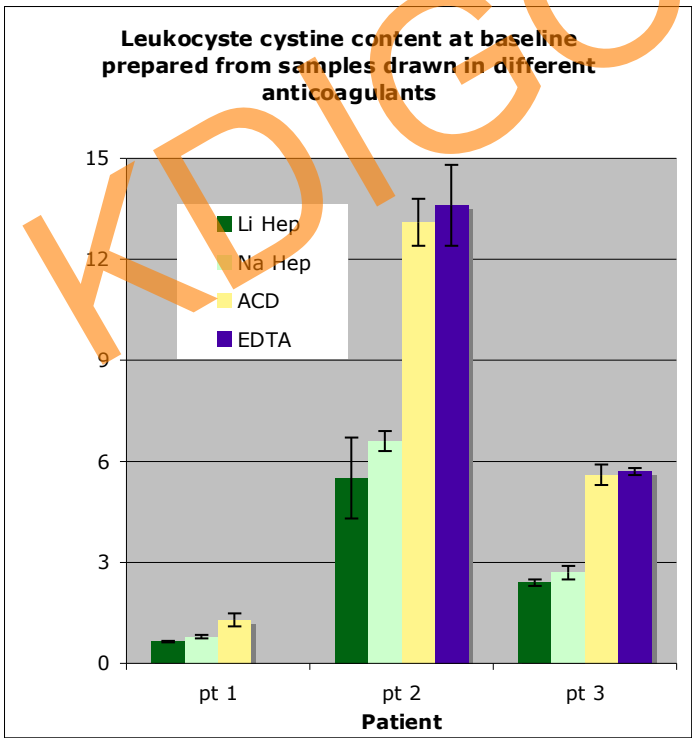
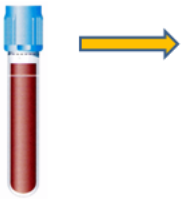


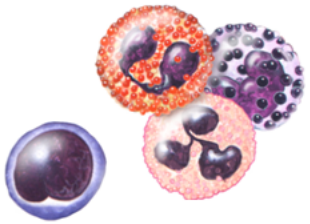
Fidler *et al.*, 2011, unpublished

new denominators: alternative normalization

Discovery of peptides from lysosomal proteins endogenous in leukocytes



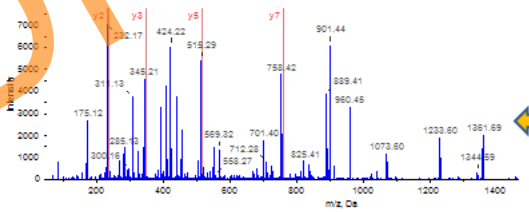
Whole blood



Mixed leukocyte extraction

White blood cell lysis /protein precipitation/resolubilization

Trypsin proteolysis



N	% protein Coverage	Protein Name	Peptides(95%)
1	51.2	fibronectin 1 isoform 5 preproprotein [Homo sapiens]	69
2	56.7	talin 1 [Homo sapiens]	62
39	**37.5**	**elastase 2, neutrophil preproprotein [Homo sapiens]**	**9**
40	**41.4**	**azurocidin 1 preproprotein [Homo sapiens]**	**5**
91	**14.8**	**proteinase 3 (serine proteinase, neutrophil, Wegener granulomatosis autoantigen) [Homo sapiens]**	**2**
181	3.3	transaldolase 1 [Homo sapiens]	1

Peptides for nearly 180 proteins were matched with over 95% confidence using Protein Pilot (AB Sciex). Peptides from 3 lysosome-localized proteins In neutrophils were found: **Elastase 2**, **Azurocidin 1**, and **Proteinase 3**.

MS/MS scans were performed on several thousand peptides from a single LC gradient run on a C18 capillary column coupled to a Q-TOF.

Liquid chromatography coupled to MS/MS (Qstar Elite, AB Sciex)

alternative cell types for cystine assay

Non-hematologic cell types

Prospect: Renal tubular epithelial cells isolated from urine:
Advantages: May better reflect long-term medication exposure
Readily accessible cell type; May reflect therapeutic effect at key site;
Disadvantages: New reference ranges req'd. Could not use post renal transplant.
?Volume req'd, ?effect of treatment PK/urine [cysteamine]
Approach: immuno-purification.

Prospect: Buccal epithelium by cheek swab:
Advantages: Readily accessible cell type; May reflect therapeutic effect
at other tissues; could be valid post transplant. Could have home collection.
Approach: Demonstrate feasibility in heterozygotes and treated homozygotes
post rinse, 10 swipes with buccal swab, placed in SSA solution, extracted.

optimal timing of testing

Population pharmacokinetics and pharmacodynamics of cysteamine in nephropathic cystinosis patients

Naïm Bouazza[1,2*], Jean-Marc Tréluyer[1,2,3,4], Chris Ottolenghi[5], Saik Urien[1,2,4], Georges Deschenes[7], Daniel Ricquier[5], Patrick Niaudet[6] and Bernadette Chadefaux-Vekemans[5]

Orphanet Journal of Rare Diseases 2011, 6:86

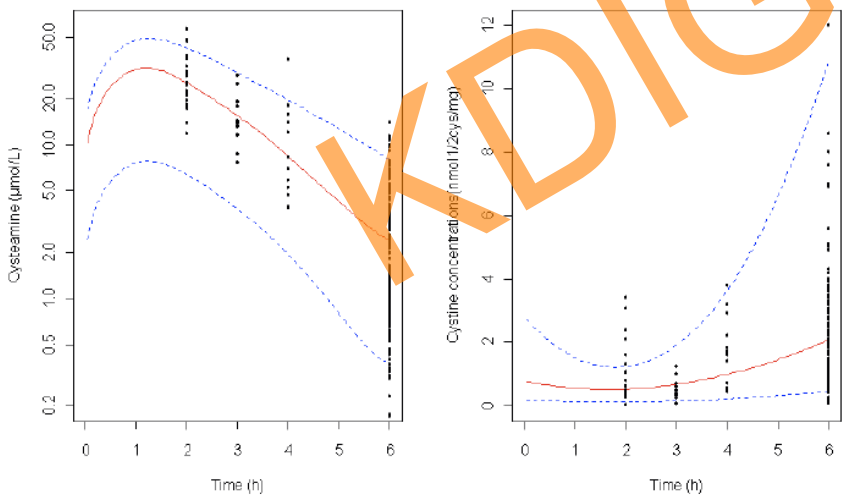


Figure 2 Evaluation of the final model: comparison between the 5th, 50th and 95th percentile obtained from 400 simulations (lines), and the observed data (o) for cysteamine concentrations standardized for a cysteamine dose of 900 mg/ day (A) and for WBC cystine levels (B).

Proposed daily dose:
80 mg/kg/d (QID): 10-17 kg,
70 mg/kg/d (QID): 17-25 kg, 60 mg/kg/d (QID): 25-40 kg,
50 mg/kg/d(QID): 40-70 kg.

Generally would presume best testing time would be trough level of drug/ highest level of cystine. But drug level most variable in terminal phase.

optimal timing of testing

A Randomized Controlled Crossover Trial with Delayed-Release Cysteamine Bitartrate in Nephropathic Cystinosis: Effectiveness on White Blood Cell Cystine Levels and Comparison of Safety

Clin J Am Soc Nephrol 7: 1112–1120, 2012.

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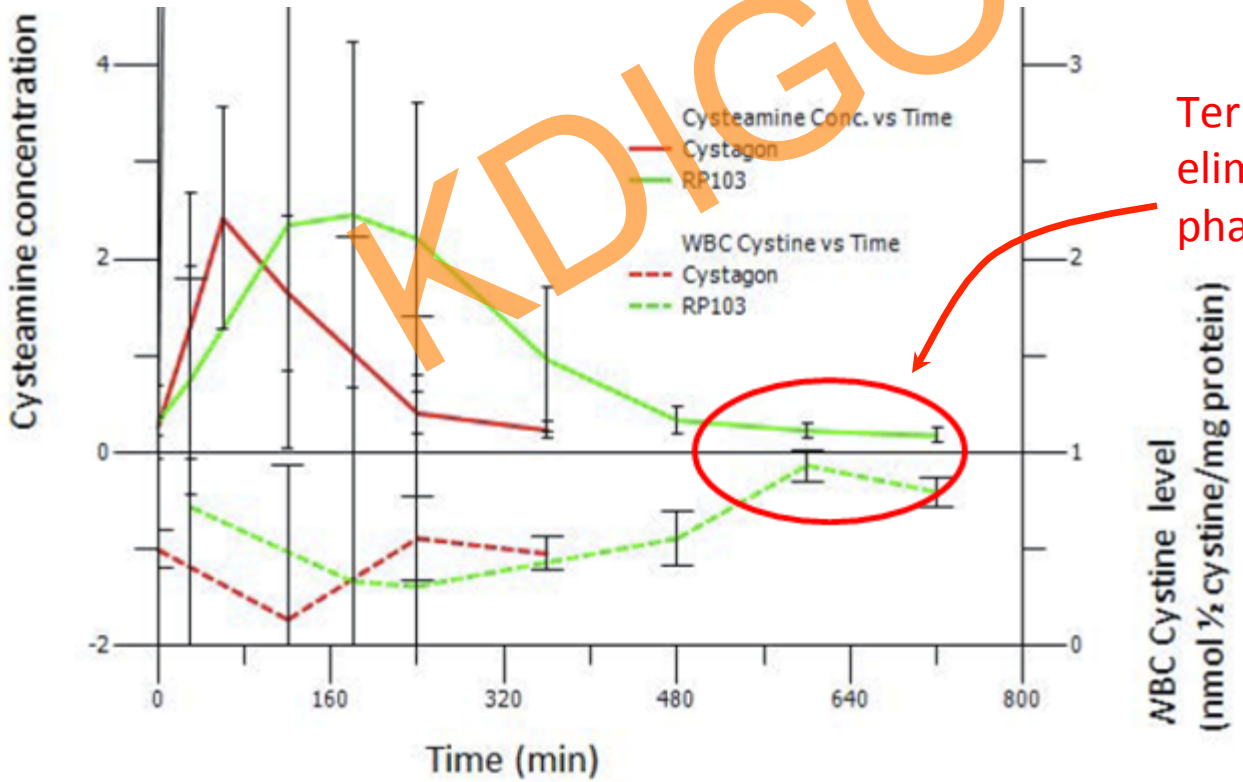

Terminal elimination phase

residual cysteamine as surrogate marker

Quality of Life is Improved and Kidney Function Preserved in Patients with Nephropathic Cystinosis Treated for 2 Years with Delayed-Release Cysteamine Bitartrate

Langman *et al.*
J Pediatr. 2014 September ; 165(3): 528–533

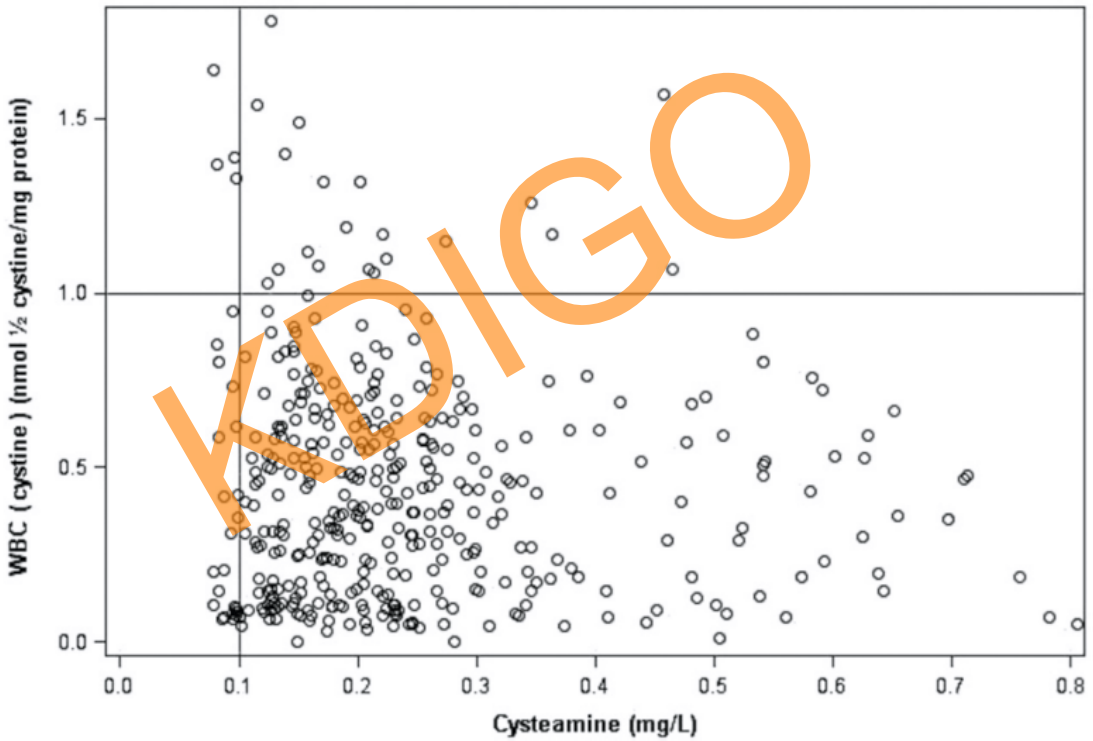

Figure 2.
WBC (cystine) vs plasma (cysteamine) for all study patients who had a WBC (cystine) ≤1 nmol/1/2 cystine/mg protein; 94.5% of measured plasma (cysteamine) values were >0.1 mg/dL when the WBC (cystine) was ≤1 nmol ½ cystine/mg protein.

residual cysteamine as surrogate marker

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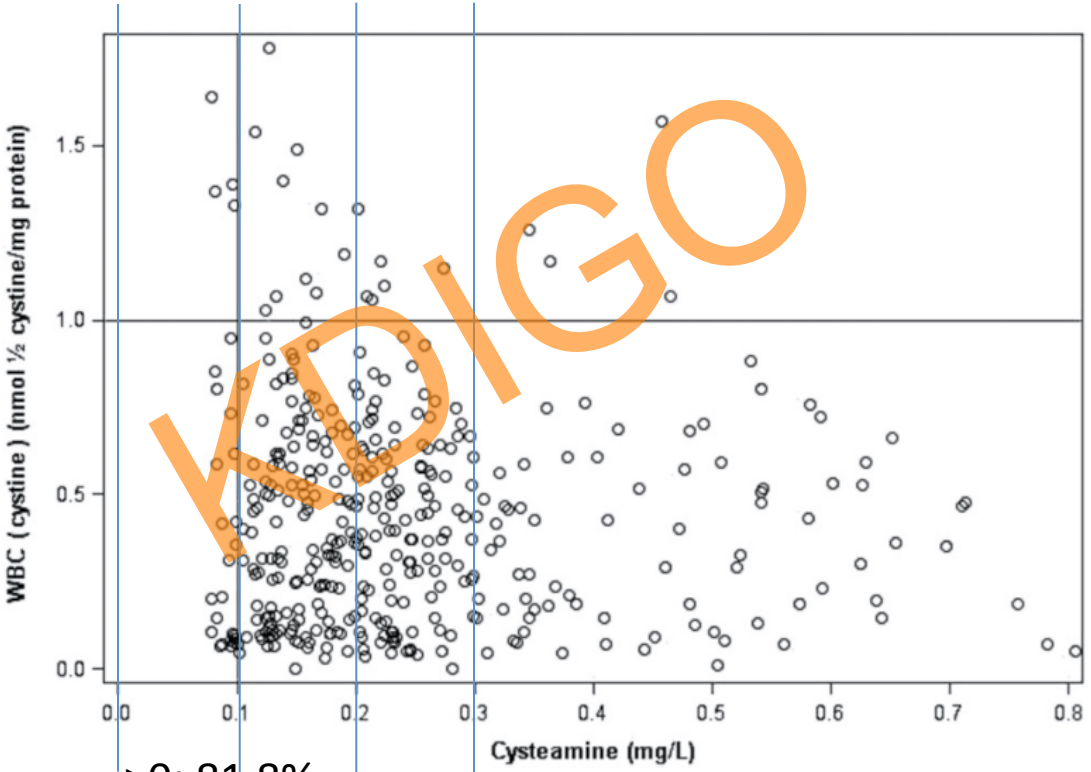

Percent with WBC ½-cystine <1 nmol/mg:

>0: 81.8%

>0.1: 94.5%

>0.2: 95.2%

>0.3: 93.75%

controversies conference

- why monitor?
- target range
- earliest treatment/ earliest measurement
- pre-symptomatic diagnosis/ screening
- sedoheptulokinase
- ctns mutations
- common mutations/uncommon mutations
- optimal technique for testing
- denominator effects
- optimal prep
- optimal storage
- alternative normalization
- alternative cell types for cystine assay
- optimal timing of testing
- residual cysteamine as surrogate marker

other questions

- Is it feasible to perform pre-symptomatic screening of cystinosis? In utero and in newborns?
- What is the optimal technique for white blood cell (WBC) isolation and storage?
- What is the optimal technique for WBC cystine measurement, including timing of the measurement?
- Are there alternatives to WBC cystine measurements to monitor cysteamine treatment (plasma cysteamine, others)?
- What is the role of cystine as a biomarker and cysteamine blood levels as a surrogate?
- **Can we measure crystal loads?**
- **Is genetic diagnosis mandatory?**
- **Is urine analysis helpful to raise the suspicion or make the diagnosis?**
- **What other biochemical monitoring should be undertaken in treated patients?**
- **What are the major clinical hints, providing high index of suspicion to diagnose cystinosis as early as possible ?**
- **What is the final decision regarding carnitine supplementation for patients post transplant? Is therapy worth the cardiovascular risk?**
- To discuss controversies of newborn screening, molecular diagnosis availability
- To discuss controversies of the time post Procysbi dose to evaluate WBC cystine reduction: 11.5 versus 12.5 hours


KDIGO