CLINICAL AND GENETIC ANALYSIS OF A COHORT OF UK CYSTINURIA PATIENTS

Hannah RHODES[1], Laura YARRAM-SMITH[2], Sarah RICE[3], Ayla TABAKSERT[4], Noel EDWARDS[3], Mark WOODWARD[1], Sarah SMITHSON[5], Charlie TOMSON[6], Gavin WELSH[1], Maggie WILLIAMS[2], David THWAITES[3], John SAYER[4]*, Richard COWARD[1]*

*[1]Academic and Children´s Renal Unit, School of Clinical Sciences, University of Bristol; [2]Bristol Genetics Laboratory, Pathology Sciences, Southmead Hospital, Bristol; [3] Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle University; [4]Institute of Genetic Medicine, International Centre for Life, Newcastle University; [5]Department of Clinical Genetics, University Hospitals Bristol; [6]Richard Bright Renal Unit, Southmead Hospital, Bristol. *Authors contributed equally*

Background

Cystinuria is an inherited renal stone disease

The inability to reabsorb cystine and dibasic amino acids by the proximal tubular cells of the kidney leads to crystalluria (*Figure 1*) and the formation of cystine calculi *(Figure 2)*.

- Causes 10% of stones in children and 1% in adults
- Usually presents before 30 years of age
- Prevalence in UK unknown
- Current treatments (*Figure 3*) are non-curative

Figure 3: International Treatment Guidelines (AUA,EAU):
- Increased fluid intake (urine output >3L/day)
- Urine alkalinisation – Potassium citrate, sodium bicarb.
- Addition of cystine-binding drug – tiopronin
- Surgical removal of problematic urinary tract stones

Figure 1: Characteristic morphology of cystine crystalluria

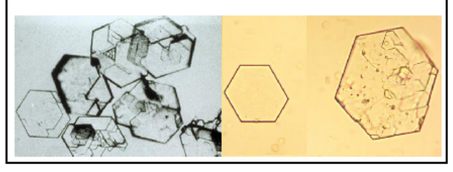


Figure 2: Cystine stones produced by adolescent patient over 3 month period

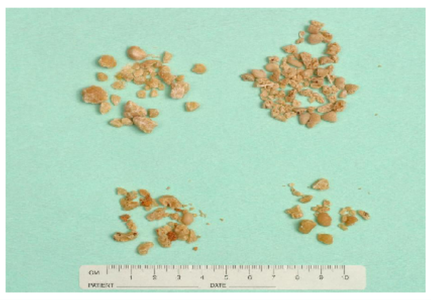


Figure 4: Cystine transport in proximal tubular cells via the rBAT/ $b^{0,+}AT$ heterodimer

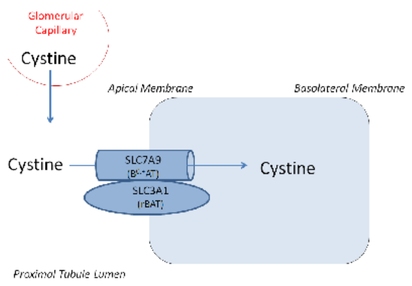


Figure 5: Nomenclature for Cystinuria Genotypes

Type AA = 2 mutated *SLC3A1* alleles
Type BB = 2 mutated *SLC7A9* alleles
Type A = 1 mutated *SLC3A1* allele
Type B = 1 mutated *SLC7A9* allele
Type AB = 1 of each mutated allele
Type AA(B) or BB(A) = more than 2 mutated alleles present

Mutations in *SLC3A1* and/or *SLC7A9* cause cystinuria

- *SLC3A1* and *SLC7A9* genes encode the two subunits of the cystine transporter, rBAT and $b^{0,+}AT$, (*Figure 4*)
- Patients with mutations in *SLC3A1* have Type A, *SLC7A9* Type B, and Type AB has been more rarely observed, see *Figure 5* for nomenclature.

Inheritance of Cystinuria

- ***SLC3A1* autosomal recessive**
- Heterozygotes of *SLC3A1* have normal urinary cystine and dibasic amino acids
- Except some heterozygotes with the duplication of exons 5-9

- ***SLC7A9* autosomal dominant with variable penetrance**
- 86% of *SLC7A9* heterozygotes have abnormal urinary dibasic amino acid levels
- Some heterozygotes develop cystine stones

Aims

1. Identify large cohort of UK cystinuria patients
2. Estimate prevalence of cystinuria
3. Analyse clinical features of cystinuria cohort
4. Undertake genetic analysis of *SLC3A1* and *SLC7A9*
5. Determine if genotype predicts disease severity

Patient cohort

- **Cystine stone(s) confirmed on chemical analysis**
- Hospitals throughout the South West and North West regions
- Detailed clinical data collected for genotype/phenotype analysis
- Population estimates from 2011 UK Census

Methods

Genetic Analysis

- **Sanger sequencing** of all coding exons and flanking intronic regions (including splice sites and branch points) of *SLC3A1* and *SLC7A9* using bidirectional automated high-throughput sequencing (Beckman NX/ABI3730), and analysed with Mutation Surveyor software Alamut (version 2.3 rev 1)
- **Multiplex Ligation-Dependent Probe Amplification** (MLPA) of coding exons utilising an in-house high-throughput automated MLPA assay (Beckman NX/Beckman CEQ8000). Probes for *SLC3A1* & *SLC7A9* as per Bisceglia et al (2010) with control P200 probe kit from MRC-Holland.

Ethics: Informed consent and ethical approval obtained: 12/SC/0456 and 11/NE/0259.
Statistics: Mann Whitney U test (2-tailed) to compare two independent groups of non-Gaussian data using Graphpad Prism v5

Results

Cohort of 76 patients with cystinuria:

- 55% male, 97% white British
- Median age at 1st stone 24 years (range 2-62 years)
- 21% aged over 40 years at presentation
- Median stone frequency per year 0.45 (range 0.06-78.2)
- 15 patients (20%) had staghorn stones
- 53 (70%) had eGFR < 90ml/min/m² (Table 2)
- 54 received medical therapy following international guidelines (*Figure 3*) of whom 27 (50%) continued to form cystine stones
- Prevalence of cystinuria ~1 in 100,000 (population size 7,886,100)

Genetic Analysis

- 125 mutated alleles identified
- 37 distinct variants detected (distribution shown in *Figure 6*)
- 12 novel mutations; 8 in *SLC3A1* and 4 in *SLC7A9* (*Figure 6)*
- 22% (27/125) are large gene rearrangements
- 20% (15/76) are homozygous for mutant alleles
- Frequency of genotypes detected is shown in *Table 1*

Table 1: Frequency of genotypes

Genotype	N°.	% of total
AA	27	36
BB	20	26
AAB	1	1
BBA	1	1
BBB	1	1
A	5	7
B	17	22
Unsolved	4	5
Total	76	

Table 2: Comparison of renal function by genotype

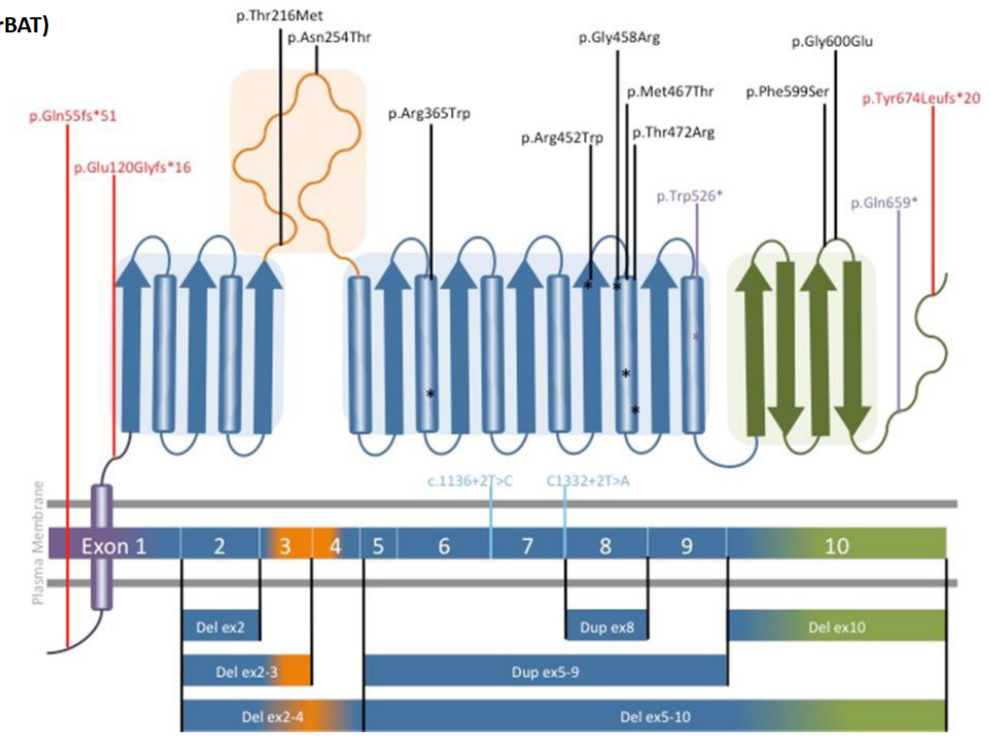
eGFR	N°.	%	AA	A	BB	B	AB	?*
>90	23	30	8		6	8		1
60-89	37	49	16	4	7	5	3	2
30-59	9	12	2		3	3		1
<30	4	5	1	1	2			
ESRD (with transplant)	3	4			2	1		

(?= unsolved)*

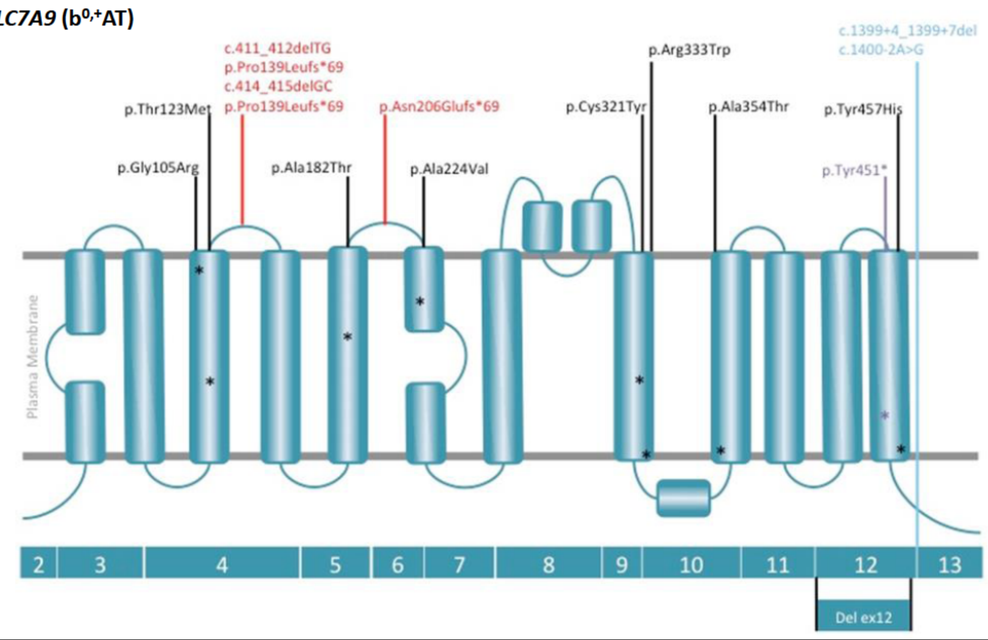
Results

Figure 6(a): Distribution of mutations detected in *SLC3A1* (rBAT) throughout all exons and protein domains. Schematic diagram of rBAT based upon a figure by Eggermann *et al.* (2012) and a homology model of the extracellular domain of rBAT based on the crystal structure of oligo-1,6-glucosidase from *Bacillus cereus* (PDB code 1UOK). The three domains of rBAT are shown in purple (TMD), blue (Domain A), orange (Domain B (subdomain)), and green (Domain C).
Figure 6(b): *SLC7A9* ($b^{0,+}AT$) mutations distributed in exons 4-6, 9, 10, and 12-13. Schematic diagram of $b^{0,+}AT$ based upon figures by Eggermann *et al.* (2012) and Yamashita *et al*. (2005), and a homology model of $b^{0,+}AT$ based on the crystal structure of AdiC, an Arginine:Agmatine Antiporter from *E.coli* (PDB code 3L1L). *Mutations are labelled as follows: Missense (black), Nonsense (purple), Frameshift (red), Splice-site (pale blue). Mutations predicted to fall within alpha helices are denoted by * of the appropriate colour.*

(a) *SLC3A1* (rBAT)



(b) *SLC7A9* ($b^{0,+}AT$)



Genotype/Phenotype Analysis

- Overlap between all the genotype groups regarding age at first stone event, see *Figure 7(a)*
- Variability both between and within the genotype groups for the level of urinary cystine at first presentation (*Figure 7(b)*)
- Renal impairment present in all genotype groups (*Table 2*)

Figure 7: Genotype-phenotype. (a) shows the age in years at first stone event for each genotyped group, (b) shows 24 hour urinary cystine at first presentation demonstrating the considerable overlap between the genotype groups.

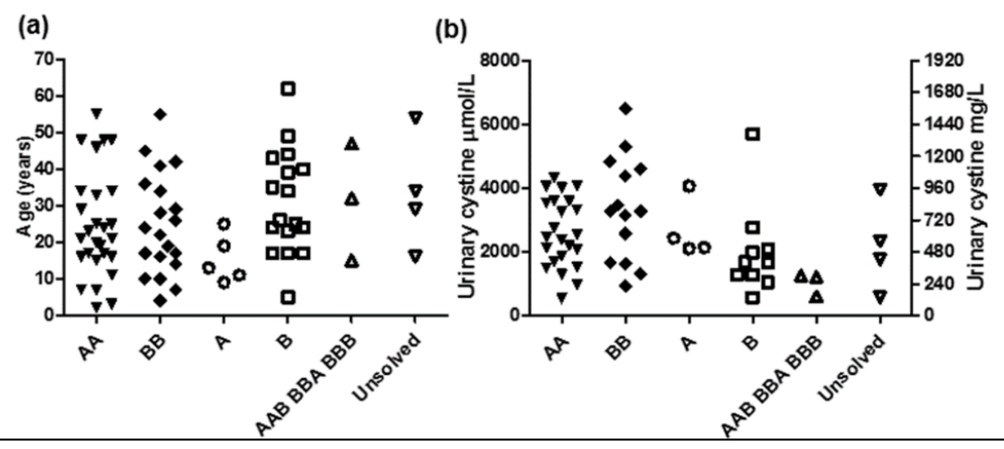


Table 3: Comparison of genotypes AA (*SLC3A1*) and BB (*SLC7A9*): no difference between the 27 type AA and 20 BB patients regarding age at first stone, stone episodes per year or renal impairment

Genotype	Median Age at 1st stone event (range)	Median stone episodes per year (range)	% with renal impairment (eGFR < 90ml/min/m²)
AA	21 (2-55)	0.44 (0 - 7.13)	70%
BB	23 (4-55)	0.48 (0.09 - 13.3)	82%
p value	0.957	0.392	1.00

Conclusions

1. **Cystine stones commonly first present in adulthood in cystinuria**
2. **Current treatment options are ineffective**
3. **No genotype-phenotype correlation in these patients**
4. **Presence of a single detectable mutation in both *SLC3A1*- and *SLC7A9*-related disease is common and sufficient for a dramatic clinical phenotype**
5. **Several patients do not have an easily identifiable mutation in the known cystinuria genes**
6. **Further sequencing may identify intronic mutations or disease-modifying SNPs**
7. **Collaboration underway for larger study via national registry**

Hannah.Rhodes@Bristol.ac.uk

52 ERA
10--SP
G) Nephrolithiasis, divalent ions and divalent ions disorders.
Hannah Rhodes
DOI: 10.3252/pso.eu.52era.2015
ePosters supported by F. Hoffmann- La Roche Ltd.
52nd ERA-EDTA CONGRESS London 2015 UNITED KINGDOM MAY 28th-31st
PosterSessionOnline.com