

Genotype-Phenotype in Egyptian Patients with Nephropathic Cystinosis

(December 2012 report)

This is the first study of the genotype of Nephropathic Cystinosis (NC) patients in Egypt and the region of North Africa. Samples of 15 Egyptian pediatric patients from 13 different families with the confirmed diagnosis of NC were obtained. Diagnosis of cystinosis was made by the detection of typical corneal cystine crystals in all patients; one patient was diagnosed by measuring PMN cystine content at 10 months of age.

Specific aims of the grant:

- A. To teach the Egyptian trainee the methodology for isolation of polymorphonuclear leukocytes (PMN) and measuring PMN intracellular cystine content for patients with NC.
- B. To examine molecular genetic testing to unravel the underlying genetic mutational defect in these patients.
- C. To study the genotype - phenotype correlation in these patients.

Methods:

- A. The trainee has trained on the cystine assay in PMN by LC-MS/MS at the Laboratory of Metabolic Diseases (UCL, Brussels). He performed and mastered the following techniques:
 - 1- Separation of PMN cells for the assay.
 - 2- Preparation of samples for LC-MS/MS.
 - 3- Measuring proteins by Lowry method.

- 4- Operating the MS instrument.
- 5- Interpretation of MS cystine results.

A. DNA samples of the 15 Egyptian patients were studied in the laboratory of pediatrics/pediatric nephrology research laboratory in the University Hospital of Leuven (UZ Leuven)/Catholic University of Leuven. All samples were first screened for the 57Kbp deletion which is common in cystinosis patients of European descent by standard multiplex PCR technique, and then direct sequencing of the ten coding exons and exon-intron interfaces of the *CTNS* gene was performed.

The trainee has performed and mastered the following:

- 1- PCR assay of the 57Kbp deletion.
- 2- Exon PCR analysis.
- 3- DNA sample preparation for sequencing.
- 4- Interpretation of sequencing results.
- 5- Using mutation prediction data base software.

Results:

- A. The trainee has performed the different techniques involved in the assay at least on 3 different occasions on normal and European NC patients.
- B. Molecular testing of the 15 Egyptian patients was performed by the trainee. None of the patients had the 57Kbp deletion mutant allele, 12 pathogenic

mutations were identified representing 27 mutant alleles, six were previously reported and six - new mutations (two frame shift, two missense (one of them could have an effect on splicing), one nonsense and one splice site mutation. Ten out of the 15 patients had homozygous mutations (Table 1).

Newly detected mutations were screened for pathogenicity using different web based software: PolyPhen2, Spliceman, BDGP (Table 2).

Discussion:

Egyptian patients have a distinct genotype compared to the patients from the North and South of Europe with an absence of the 57kb deletion and six new mutations that have not been previously reported .

The most common mutation detected in Egyptian patients is the c.829dup found in eight mutant alleles in four patients from three different families. This mutation was previously reported only in one North European patient in a heterozygous state (Table 1).

A similar study in 12 Turkish patients also reported the absence of the 57Kbp deletion, however, only two out of 12 mutations in our study were detected in the Turkish patients (c.681G>A and c.1015G>A) (Topaloglu et al. *Pediatr Nephrol* 2012).

Finally, the high rate of homozygous mutations (67%) in the Egyptian patients sheds the light on the gravity of the role of consanguineous marriage in elevating the incidence of Nephropathic Cystinosis and other inborn errors of metabolism in Egypt.

Work still to be performed:

- 1- Confirmation of the 2 detected splice site mutations by RT-PCR in mRNA samples (patients 2 and 9) (to be performed in Belgium).
- 2- Screening of the promoter region in patients with still unrevealed mutations (patients 11 and 14) (to be performed in Belgium).
- 3- Cystine assay for all Egyptian patients (to be performed in Egypt).
- 4- The detailed genotype - phenotype correlation study, writing a paper (to be performed in Egypt and in Belgium).

Table 1: Genotype-Phenotype summary of Egyptian patients with Nephropathic Cystinosis.

Proband	Family	Age in years	Sex	Onset of symptoms in months	Age at diagnosis	Dose of Cysteamine (mg/kg/day)	Renal status	Extrarenal manifestations	Mutant allele1	Mutant allele2	Reference to known mutation
1	1	3	M	6	20	30	Renal impairment	no	c.829dup	c.829dup	<i>Besouw et al., 2012</i>
2	2	5	F	8	14	30	Fanconi syndrome	no	IVS3+5g>t ^a	IVS3+5g>t ^a	-----
3	3	3	M	9	22	30	Fanconi syndrome	no	c.922G>A	c.922G>A	<i>Shotelerusk et al., 1998</i>
4 ^b	4	15	F	12	108	25	Fanconi syndrome	no	809-811del	809-811del	<i>Attard et al., 1999</i>
5	5	16	F	6	60	25	ESRD	hypothyroidism	c.829dup	c.829dup	<i>Besouw et al., 2012</i>
6	5	6	M	5	6	30	Fanconi syndrome	no	c.829dup	c.829dup	<i>Besouw et al., 2012</i>
7	6	7.5	M	5	9	30	Fanconi syndrome	no	c.15G>A	c.15G>A	<i>Kalatzis et al., 2002</i>
8	7	5	M	6	14	40	Fanconi syndrome	no	c.681G>A	c.681G>A	<i>Attard et al., 1999</i>
9 ^c	8	8	M	6	10	-----	ESRD	no	260-261del ^a	c.560A>G ^a	-----
10	9	4	M	7	15	35	Fanconi syndrome	no	c.1015G>A	c.1015G>A	<i>Shotelerusk et al., 1998</i>
11	10	4.5	F	10	30	30	Fanconi syndrome	no	c.1084G>A ^a	ND	-----
12	11	11	F	5	30	15	ESRD	hypothyroidism	c.734G>A ^a	c.1033insG ^a	-----
13	11	6.5	F	4	5	45	Fanconi syndrome	hypothyroidism	c.734G>A ^a	c.1033insG ^a	-----
14	12	3.5	M	8	14	45	Fanconi syndrome	no	ND	ND	-----
15	13	4	M	6	36	45	Renal impairment	no	c.829dup	c.829dup	<i>Besouw et al., 2012</i>

^a, New mutation; ^b, Patient had renal transplantation 1 year ago; ^c, Patient died Dec 2012; ESRD, end stage renal disease; ND, not detected.

Table 2: Predicted effects of new CTNS variants

Variant	Prediction						
Deletions or insertions							
c.260-261delTT; p.I123FX123	Frame shift						
c.1033insG; p.D364FX364	Frame shift						
Nonsense							
c.734G>A; p.W245FX245	Nonsense						
Missense							
	PMut			Mutation taster		PolyPhen2	
	NN output	Reliability	Result	Probability	Result	Score	Result
c.1084G>A; p.G362R	0.8395	6	Pathogenic	0.996	Pathogenic	0.989	Probably damaging
c.560A>G ^a ; p.K187R	0.0145	9	Neutral	0.952	Pathogenic	0.743	Possibly damaging
Probable splicing variants							
	Spliceman		BDGP		Automated splice site analysis		
	Ranking	Result	Score	Result	Final/initial binding	Result	
IVS3+5g>t	0.85	Pathogenic	1.0>0.67	Pathogenic	6.6%	Pathogenic	
c.560A>G ^a	0.55	Could be pathogenic	1.0>0.99	Neutral	20.4%	Pathogenic	

^a, A missense mutation and a probable splicing mutation; **PMut**: <http://mmb2.pcb.ub.es:8080/PMut/>; **Mutation taster**: www.mutationtaster.org/; **PolyPhen2**: genetics.bwh.harvard.edu/pph2/; **Spliceman**: fairbrother.biomed.brown.edu/spliceman/; **BDGP**: www.fruitfly.org/seq_tools/splice.html; **Automated splice site analysis**: <https://splice.uwo.ca/>