MP24

Identification of a novel genomic mutations through a next-generation sequence among 51 Japanese Cystinuria patients (Look for a missing peace of Genotype Criteria?)

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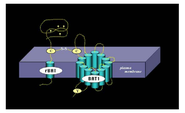
Back Grounds: Unique Characteristics of Japanese Cystinuria

What is Cystinuria?

- Autosomal recessive disorder
- Cause recurrent Urinary Stone
- Impair transport of Cystine and dibasic amino acids (Lys, Arg , Orn)
- Defect in Cystine transporter BAT1(*SLC7A9*) and rBAT (*SLC3A1*) will cause Cystinuria



Incidence

1 /16,000 Japanese
1/ 15,000 United State
1/2500 Lybian Jewish

30y history of Cystinuria Study

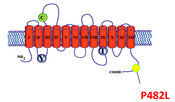
- 1983 Analyzed 110,000 Urine sample and identified 39 Cystinuria patients Ito H. J Urol. 1983 May;129(5):1012-4.
- 1999 Cloning of Cystine transporter; *BAT1* in mouse Chairoungdua A,et al., J Biol Chem. 1999 Oct 8;274(41):28845-8.
- 2000 Identified Mutation in Cystine transporter; *rBAT* Egoshi KI, et al., Kidney Int. 2000 Jan;57(1):25-32.
- 2001 Cloning of Cystine transporter; *BAT1* in Human Mizoguchi K, et al., Kidney Int. 2001 May;59(5):1821-33.
- 2002 Functional analysis of Cystine transporter; *rBAT* mutation Ishihara M,et al., Nephron. 2002 Jun;91(2):276-80.
- 2006 Identification of Japanese Unique mutation in *BAT1*; P482L Shigeta Y , Sakamoto S et al., Kidney Int. 2006 Apr;69(7):1198-206.
- 2009 Role of *BAT1*-C terminus in translocation of the transporter Sakamoto S et al., Biochem J. 2009 Jan 15;417(2):441-8
- 2012 Genomic Analysis of 92 Japanese Cystinuria patients ; need for novel classification Sakamoto S et al., Japanese Urological Association Annual Meeting
- 2013 Collaboration Study of Korean Cystinuria patients with Seoul National University; Prof. Hae Il Cheong
- 2018 Collaboration study with Malaysia(Malaya University), Taiwan (Chang Gung University), China(Chinese University of Hong Kong), and Thai (Chulalongkorn University)

Japanese unique mutation ; P482L?

1st analysis of Japanese 42 Cystinuria Patients

BAT1 mutations	
P482L(homo)	22
P482L(hetero)	6
G195R(hetero) / P482L (hetero)	1
R333W(hetero) / P482L (hetero)	1
R333Q(hetero) / P482L (hetero)	1
N227D(hetero)	1
1105delA(hetero)	2
W69stop(hetero) / 1105delA(hetero)	1
total	35

31/35(90%) of BAT1 mutations was P482L



No report in the world
=Japanese unique mutation

Shigeta et al., Kidney Inter. 2006 ; 69(7):1198-206

Clinical Question of Cystinuria

Genotype classification of Cystinuria

Type A: two mutations in *SLC3A1* (rBAT)

Type B: two mutations in *SLC7A9* (BAT1)

Type AB: one mutation on *SLC3A1* and one mutation in *SLC7A9*

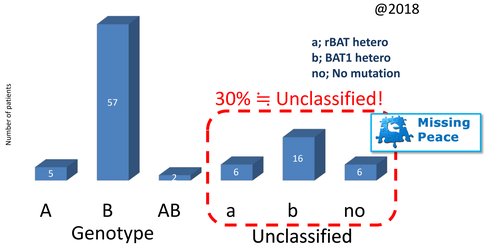
Luca D. et al., J. Am. Soc. Nephro., 2002

Based on autosomal recessive inheritance

Current Genotype of Japanese Cystinuria

Based on direct sequence of 100 Japanese patients

@2018



Unsolved problem of Cystinuria

To identified the reason why 30% of patients has only one or less mutation.

Objectives and Methods

Hypothesis

30% ≒ Unclassified!

1. RNA Splicing? → New mutation in exon-Intron boundary

2 Missed by Direct Seq → New mutation in exon by Next Seq

Method

- Purpose : Next generation sequence(Next Seq) of rBAT(*SLC3A1*) and BAT1 (*SCL7A9*)
- Material : DNA from 51Cystinuria patients previously analyzed by direct sequence.
- Method : Exon＋Exon-Intron boundary sequnce of rBAT/BAT1, AGT1 and 10 stone related transporters

1. Construction of panel by IDT.
2. Makes libraries（KAPA Hyper Plus library Kit ）
3. Capture of the panel
4. Run the sequence using next generation sequence from illumine(NextSeq500 sequencer)
5.Sequence coding region and intron-exon boundary of targeted genes
6. Data analysis(home made pipeline by GATK and VarScan

Results

Table 1. Result of selected 14 patients with novel mutations or no mutation among 51 Cystinuria patients by Next Seq

Red: New Mutations

PT Number	rBAT Direct Seq	BAT1 Direct Seq	rBAT Next Seq	BAT1 Next Seq	Genotype Direct	Genotype Next	Novel mutation	Genotype Change
2	No mutation			c.873+2dupT [hetero]/p.Thr204fs [hetero]	No mutation	B	+	+
59	No mutation		No mutation		No mutation	No mutation	-	-
28	No mutation		No mutation		No mutation	No mutation	-	-
11		p.Pro482Leu [hetero]		p.Pro482Leu [hetero]/p.Val340fs [hetero]	b	B	+	+
17		p.Pro483Leu [hetero]		p.Pro482Leu [hetero]/p.Val340fs [hetero]	b	B	+	+
41		p.Pro482Leu [hetero]		p.Pro482Leu [hetero]/p.Val340fs [hetero]	b	B	+	+
56		p.Pro482Leu [hetero]/p.Gly73Arg [hetero]	p.Ile105Val [hetero]	p.Pro482Leu [hetero]/p.Gly73Arg [hetero	B	AB	+	+
58	p.Asn442fs [hetero]		p.Asn442fs [hetero]/Exon1 del [hetero]		a	A	+	+
60		p.Pro482Leu [hetero]		p.Pro482Leu [hetero]/Exon9 del [hetero]	b	B	+	+
61		A354T(hetero)G1148A		p.Ala354Thr [hetero]/p.Trp69* [hetero]	b	B	+	+
63		p.Pro482Leu [hetero]		p.Pro482Leu [hetero]/p.Asn227Asp [hetero]	b	B	+	+
68		p.Pro482Leu [homo]		p.Pro482Leu [homo]/Exon2-13 del [hetero]	B	B	+	-
71	p.Tyr371* [hetero]		p.Tyr371* [hetero]/c.1500+1G>A [hetero]		a	A	+	+
76		p.Pro482Leu [hetero]/p.Ser446Arg [hetero]	Exon4, 8-10 del [hetero]	p.Pro482Leu [hetero]/p.Ser446Arg [hetero]	B	AB	+	+
							12/51	11/51

Table 2. List of novel mutations by Next seq

	rBAT	BAT1
Intron	c.1500+1G>A [hetero]	c.873+2dupT [hetero]
Exon	p.Ile105Val [hetero]	Exon2−13 del [hetero]
	Exon4, 8−10 del [hetero]	Exon9 del [hetero]
	Exon1 del [hetero]	p.Trp69* [hetero]
		p.Asn227Asp [hetero]
		p.Thr204fs [hetero]
		p.Val340fs [hetero]

Figure 1. Genotype Classification

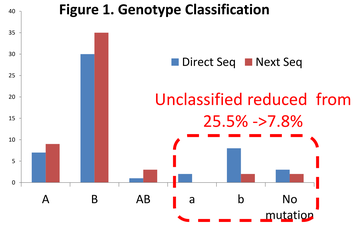


Figure 2. Cystine Concentration based on Genotype / Mutants

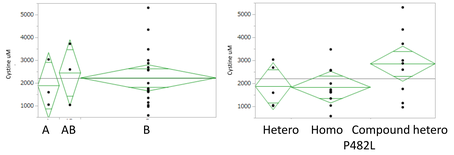
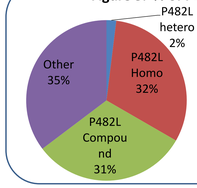


Figure 3. % of P482L related mutations

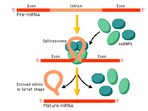


65%
is P482L related mutation
In Japanese Patients
by Next Seq

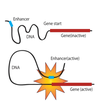
Discussion

The reason for 30% of unclassified Genotype in Japanese Cystinuria

1 Mutation in Intron-exon boundary

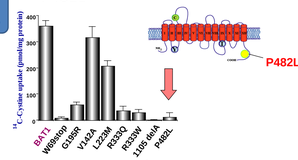


1. Affect Splicing



2. Affect Enhancer

2 P482L



P482L is strong enough to cause Cystinuria by a single mutation

3

Mutation in Unknown Gene

How to explain 2 no mutation cases ?

Conclusion

- Current data may represents that not only mutation in exon but also mutation in Intron-exon boundary of rBAT(SLC3A1)/BAT1(SCL7A9) may contribute to the pathogenesis of Cystinuria.
- Next Seq may be a power full tool to determine mutations in Cystinuria patients.

Future direction

- Determine the transcriptional level of intron-exon boundary mutants in SLC7A9 and SLC13A28 (possibly by WBC).
- Study the further genomic analysis of Cystinuria patient's genome by next generation sequence (Get a big grant!).
- International collaboration to determine the global landscape of Cystinuria mutation.

If interested in collaboration, please E mail to rbatbat1@gmail.com or Scan

2018 AUA annual meeting
COI: Author Shinichi Sakamoto declare no financial support for this presentation

WE ARE RECRUITING!

On going international collaboration

Thai, Korea, Malaysia, Hongkong, Taiwan and U.S.