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URINARY AMINO ACID PATTERNS IN CYSTINURIC FAMILIES

TOMOAKI KATO and JUNICHI SUGIURA

Department of Pediatrics, Nagoya University School of Medicine
(Director: Associate Prof. Sakae Suzuki)

ABSTRACT

The urinary amino acid patterns in parents of four cystine stone formers were investigated in order to determine the genetic types of the cystinuric families. The concentrations of urinary cystine, lysine, arginine and ornithine in the subjects were compared with those in heterozygotes of cystinuria reported by Harris and Rosenberg.

All of the subjects except one who excreted the amino acids in the range between normal individuals and incompletely recessive carriers showed the characteristics of incompletely recessive heterozygotes defined by Harris.

The urinary amino acid values of the subjects lay with a wide overlap in the extent between type I and type III heterozygotes, or type III and type II heterozygotes defined by Rosenberg. One individual excreted the compounds in a more extensive range which included all of the three groups of heterozygotes.

INTRODUCTION

Harris and his coworkers[1)2)] first recognized the genetic heterogeneity of cystinuria and classified the disease into two types, 'recessive' and 'incompletely recessive', according to excretion patterns of urinary cystine and dibasic amino acids in carriers. There was no detectable abnormality of the urinary amino acids in the former group, whereas detectable abnormalities of the urinary compounds could be found in the incompletely recessive families.

Thier *et al.*[3)] and Rosenberg *et al.*[4)] further subdivided the disease into three genetic types. Type I cystinuria, which corresponds to the recessive type of Harris, was characterized by the absence of active transport of cystine, lysine and arginine in gut mucosa in homozygotes and by normal amounts of the amino acids in urine in heterozygotes. In type II cystinuria, the intestinal transport of cystine was absent but that of lysine remained in homozygotes and heterozygotes excreted markedly increased amounts of all amino acids. In type III, active transport of cystine, lysine and arginine in jejunal mucosa was present in homozygotes and the amino acid excretion in heterozygotes was moderately increased. Type II and type III are subgroups of incompletely recessive cystinuria. Rosenberg and his coworkers[5)] described that

加藤智昭，杉浦潤一
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the three types of cystinuria could be classified only by measurement of the urinary amino acids in carriers, demonstrating significant differences of the excretions among heterozygotes of the three groups. On the basis of the excretion pattern of cystinuric families, these workers[5][6] found three unusual families in which two types of heterozygotes were demonstrated within a single pedigree, and their genetic types were I-II, II-III and I-III, respectively. The double heterozygous subjects were phenotypically indistinguishable from homozygotes of genotype I-I, II-II, or III-III.

We have studied genetic types of cystinuric families, according to the urinary amino acid excretion. However, it was not possible to obtain the three groups of heterozygotes demonstrated by Rosenberg *et al.* Our observations[7] showed that the urinary amino acid values of the heterozygous subjects of the families lay in a continuous range between the normal range and the upper limit of type II heterozygotes.

In the present work, eight heterozygotes of cystinuria were studied to confirm if their genetic types could be identified only by analysis of their urinary amino acid excretion.

METHODS

Four cystinuric families were selected from previously described pedigrees[7]. The stone formers (K.M., S.N., Y.B. and T.K.) of the families were characteristic of cystinuric homozygotes as follows. Their urine specimens gave a positive reaction with the cyanide-nitroprusside reagent and showed an increased excretion of dibasic amino acids which were detected by two dimensional thin-layer chromatography. Quantitative amounts of their urinary cystine and dibasic amino acids or their amino acid clearances agreed with the values in homozygotes of cystinuria demonstrated by Harris[2] or by Crawhall[8].

Eight parents of the stone formers and two healthy volunteers with no parsonal or family history of kidney stone or renal disease were studied as the heterozygotes of cystinuria and the control subjects.

First morning, fasting urine samples were collected from the subjects. Urine samples were obtained five to ten times from each subject. All urine specimens were kept frozen at $-20°C$ until analyzed.

Urinary amino acids were measured by an automatic amino acid analyzer (HITACHI KLA-3). The reproducibility of this method was tested for standard amino acid solutions (Table 1).

The urinary amino acids were expressed as milligrams of amino acid per gram of creatinine. Creatinine was determined by the method of Folin[9].

In order to determine the genetic types of the cystinuric families, the values of urinary amino acids in the subjects were compared with those in heterozygotes of cystinuria defined by Harris[2] or by Rosenberg[6].

TABLE 1. Reproducibility of the Results using 1.0 μ Mole of Each Amino Acid of the Standard Amino Acid Solution

	N	Mean	SD (%)
Cystine	5	16.8	2.09
Lysine	6	18.4	1.91
Arginine	6	16.2	1.31
Ornithine	6	22.8	1.28

RESULTS

The concentrations of urinary cystine, lysine, arginine and ornithine in the parents of the stone formers and in the normal individuals are presented in Table 2 and their excretion patterns are shown in Figs. 1-5.

The results in Table 2 show that there is a wide intraindividual variation in the values of the amino acids in the heterozygous subjects. Two normal individuals always excreted normal amounts of cystine and the dibasic amino acids. The parents of the stone formers except K. M.'s mother excreted the

TABLE 2. The Urinary Excretion of Cystine and Dibasic Amino Acids in Parents of Four Cystine Stone Formers and in Normal Individuals

Subject	Age	Cystine	Lysine	Arginine	Ornithine
		(mg. amino acid/g. creatinine)			
K.M.'s Father	42	17.7	300	5.08	9.35
		59.9	248	11.6	5.91
		30.8	139	6.98	5.42
		60.4	342	12.2	11.8
		63.8	268	10.2	14.3
		39.7	140	8.62	5.55
		73.8	284	11.7	11.2
		61.8	23.7	1.61	6.76
		54.4	226	6.32	11.3
		26.5	105	3.16	2.52
K.M.'s Mother	38	29.1	108	1.61	2.12
		20.1	223	5.88	4.44
		8.17	37.6	2.90	3.56
		104	33.4	1.34	1.05
		56.0	27.0	4.41	2.06
		11.5	68.0	1.46	1.23
		30.7	17.5	0.469	2.04
		28.9	77.0	3.10	1.51
		18.2	87.9	2.08	2.30
		18.4	64.2	2.19	1.17
S.N.'s Father	41	85.3	376	15.1	11.4
		86.0	341	11.6	15.9
		160	841	43.4	23.8
		259	661	36.1	44.9
		185	491	22.6	35.0
		171	454	13.5	27.5
		128	372	14.5	16.7
		177	572	26.3	35.1
		129	371	10.6	23.1
		139	676	28.7	50.0

TABLE 2. (Continued)

Subject	Age	Cystine	Lysine	Arginine	Ornithine
		(mg. amino acid/g. creatinine)			
S.N.'s Mother	36	43.3	255	5.91	5.30
		32.5	172	9.20	3.03
		114	549	28.1	17.8
		97.0	285	17.0	11.4
		116	506	23.1	24.3
		202	837	49.2	41.2
		69.6	278	14.7	26.0
		178	529	24.5	30.3
		199	1150	66.6	80.5
		11.1	265	7.14	25.2
Y.B.'s Father	36	92.1	513	27.7	59.6
		184	367	14.8	19.4
		362	892	55.3	64.4
		71.6	650	35.5	69.6
		129	528	19.8	21.1
		72.9	494	22.1	37.4
Y.B.'s Mother	35	114	751	23.7	22.2
		126	325	9.79	8.55
		30.4	531	17.5	24.8
		34.2	288	13.2	15.2
		53.6	351	9.18	12.3
T.K.'s Father	32	17.5	77.2	2.78	1.96
		43.6	336	7.90	3.26
		68.8	393	16.4	11.6
		32.5	298	8.57	9.81
		83.7	479	14.8	32.7
		124	349	14.7	19.2
		274	982	51.2	72.7
		92.7	322	9.58	13.6
		223	639	18.6	64.9
		139	563	40.1	98.0
T.K.'s Mother	26	121	218	8.61	5.53
		111	220	15.4	7.39
		186	1020	63.4	59.4
		73.1	333	21.7	6.34
		316	1480	132	129
		286	1750	171	172
		333	1650	145	190
		219	811	53.8	73.4
		131	935	123	97.1
		303	670	34.4	45.4
Control 1 (Male)	32	14.8	58.5	5.21	0.974
		7.59	16.2	4.38	1.31
		9.61	30.0	2.81	1.02
		6.52	35.3	4.12	1.09
		11.8	40.0	3.81	1.39
		11.9	21.4	4.51	1.52
		10.0	35.1	4.34	1.49
Control 2 (Female)	28	11.1	34.3	0.937	1.01
		12.1	39.5	2.47	2.74
		13.1	53.0	0.992	4.25
		12.4	65.5	0.462	4.00
		14.1	70.1	0.934	0.968
		23.1	50.3	1.34	1.95

excesses of the amino acids. Their excretion patterns are characteristic of incompletely recessive heterozygotes defined by Harris. The urinary amino acid values of K.M.'s mother lay in the range between the normal group and

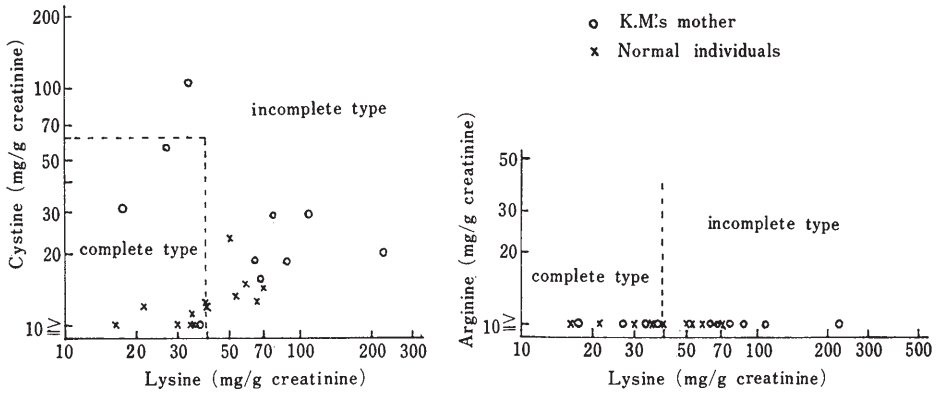

FIG. 1. The excretion pattern of cystine, lysine and arginine in K. M.'s mother and in normal individuals. The results are compared with those in heterozygotes described by Harris[2].

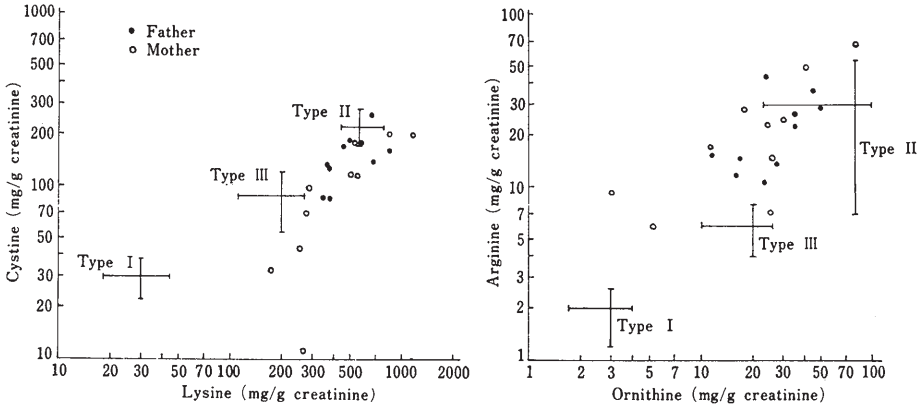

FIG. 2. The excretion pattern of cystine and dibasic amino acids in parents of S.N. The results are compared with those in heterozygotes described by Rosenberg[6].

incompletely recessive carriers (Fig. 1).

The excretions of cystine and the dibasic amino acids in the heterozygous subjects are compared with those in heterozygotes demonstrated by Rosenberg in Figs. 2-5. In the parents of S. N. and K. M., the urinary amino acid levels fell in the range between type II and type III heterozygotes, and type I and type III heterozygotes, respectively (Figs. 2, 3). The amounts of the urinary amino acids of Y. B.'s mother show the intermediate levels between type II and type III carriers, whereas Y. B.'s father shows an excretion pattern relatively typical of type II heterozygotes (Fig. 4).

The excretions of the compounds of T. K.'s father ranged from the normal range to the upper limit of type II heterozygotes and the values of T. K.'s

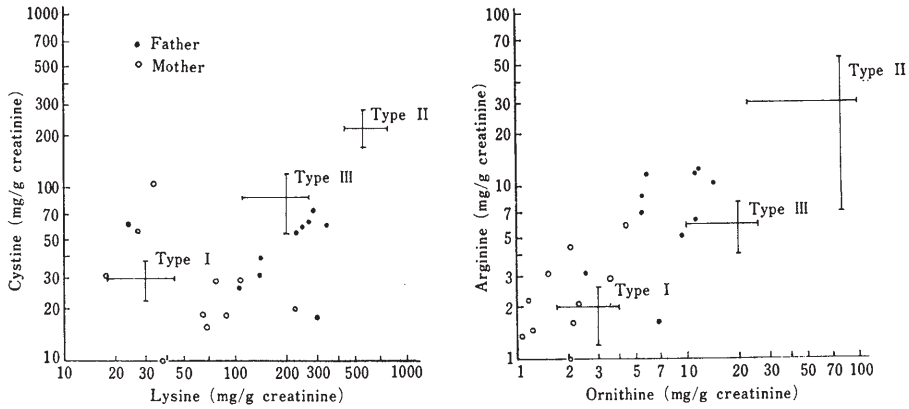

FIG. 3. The excretion pattern of cystine and dibasic amino acids in parents of K. M. The results are compared with those in heterozygotes of Rosenberg.

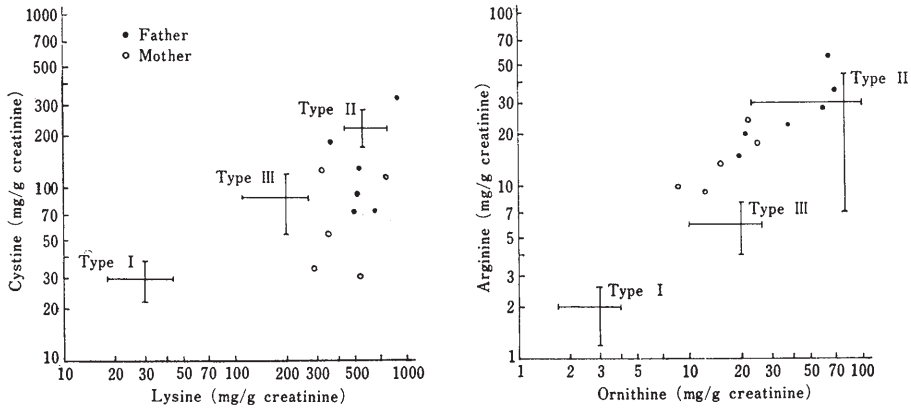

FIG. 4. The excretion pattern of cystine and dibasic amino acids in parents of Y. B. The results are compared with those in heterozygotes of Rosenberg.

mother ranged from the upper limit of type III heterozygotes to the lower limit of homozygotes (Fig. 5).

DISCUSSION

In the present studies, we have examined more than five different urine samples from each subject and observed that different urine samples showed different values of the amino acids. The results did not accord with the previous findings described by Harris or by Rosenberg.

In our heterozygous subjects, there was a wide intraindividual variation in the levels of the urinary cystine and dibasic amino acids. For example, the amounts of lysine T. K.'s father excreted were 77.2 mg. to 982 mg. per gram

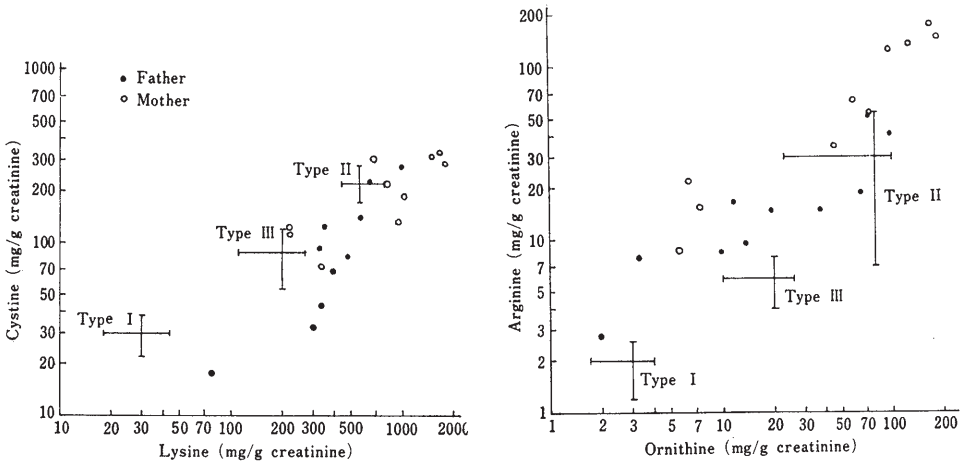

FIG. 5. The excretion pattern of cystine and dibasic amino acids in parents of T. K. The results are compared with those in heterozygotes of Rosenberg.

of creatinine, which were the levels the heterozygotes of all of the three groups excreted. The amino acid values of K. M.'s mother fell in the extent between the normal individuals and incompletely recessive carriers defined by Harris. In most of our subjects, the amounts of the amino acids excreted lay in the range with a wide area of overlap between type I and type III groups, or type III and type II heterozygotes defined by Rosenberg *et al.*

Similar findings were observed by Crawhall and his associates[10)11)]. They described that the amino acid excretion in parents of cystine stone formers lay in the range between the upper limit of normal individuals and the lower limit of homozygotes and there was no clear demarcation between normal individuals and incompletely recessive carriers on the basis of the urinary amino acid excretion.

The classification demonstrated by Rosenberg *et al.*[4)] was based on *in vitro* studies of intestinal transport of cystine and dibasic amino acids in homozygotes. However, these workers[5)] described that there were significant differences of the amino acids in urine among type I, type II and type III heterozygotes and the three types of cystinuria could be classified only by analysis of the urinary excretion of the four amino acids in heterozygotes.

The intestinal transport mechanism of the double heterozygotes defined by Rosenberg[6)] was indistinguishable from that of type I homozygotes. Rosenberg indicated that the results of oral cystine tolerance test of type I-III double heterozygotes were midway between those of type I homozygotes and type III homozygotes. However, the results of the cystine tolerance test in the double heterozygotes of remainder types were not shown. Therefore, the double heterozygotes should be identified only by measurement of the

urinary amino acids in parents of cystinuric patients.

If the three types of cystinuria can not be identified on the basis of the excretion pattern in heterozygotes, it is not possible to detect the double heterozygous subjects of cystinuria.

The present observations suggest that it is difficult to classify the heterozygotes of the disease only by analysis of their amino acid excretion patterns.

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REFERENCES

1) Harris, H., Mittwoch, U., Robson, E. B. and Warren, F. L., The pattern of amino-acid excretion in cystinuria, *Ann. Hum. Genet., Lond.*, **19**, 196, 1955.
2) Harris, H., Mittwoch, U., Robson, E. B. and Warren, F. L., Phenotypes and genotypes in cystinuria, *Ann. Hum. Genet., Lond.*, **20**, 57, 1955.
3) Thier, S. O., Segal, S., Fox, M., Blair, A. and Rosenberg, L. E., Cystinuria: defective intestinal transport of dibasic amino acids and cystine, *J. Clin. Invest.*, **44**, 442, 1965.
4) Rosenberg, L. E., Douning, S., Durant, J. L. and Segal, S., Cystinuria: biochemical evidence for three genetically distinct diseases, *J. Clin. Invest.*, **45**, 365, 1966.
5) Rosenberg, L. E., Durant, J. L. and Albrecht, I., Genetic heterogeneity in cystinuria: evidence for allelism, *Trans. Ass. Amer. Physicians*, **79**, 284, 1966.
6) Rosenberg, L. E., Cystinuria: genetic heterogeneity and allelism, *Science*, **154**, 1341, 1966.
7) Kato, T. and Sugiura, J., Genotype in cystinuria, *Pediatrics (Jap.)*, **14**, 369, 1973.
8) Crawhall, J. C., Scowen, E. F., Thompson, C. J. and Watts, R. W. E., The renal clearance of amino acids in cystinuria, *J. Clin. Invest.*, **46**, 1162, 1967.
9) Folin, O., On the determination of creatinine and creatine in urine, *J. Biol. Chem.*, **17**, 469, 1914.
10) Crawhall, J. C. and Watts, R. W. E., Cystinuria, *Amer. J. Med.*, **45**, 736, 1968.
11) Crawhall, J. C., Purkiss, P., Watts, R. W. E. and Young, E. P., The excretion of amino acids by cystinuric patients and their relatives, *Ann. Hum. Genet., Lond.*, **33**, 149, 1969.