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Familial Cystinuria in Ioannina District (Greece) Diagnosis and Treatment

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Summary. The "Urocystin Test" has been used as a screening procedure for the diagnosis of the incidence of cystinuria in Ioannina District (Northwest Greece). From the 210 investigated urine samples, eight were positive and four of them were also L-cystine stone formers. All positive cases belong to two cystinuric families. The pedigree in village "Kato-Lapsista" is a genetic type of a "completely recessive cystinuria" while the other one in "Marmara" is the type of an "incompletely recessive cystinuria". All patients with L-cystine urolithiasis, except one child, were treated with the drug Thiola.

Key words: Cystinuria, Urocystin Test, α-mercaptopropionyl-glycine.

Introduction

Cystinuria is an inherited metabolic disease. It is characterized by the elimination in the urine of increased amounts of L-cystine and other amino-acids such as ornithine, lysine and arginine.

Since L-cystine, because of its physicochemical properties, is less soluble than the other aminoacids, the aminoaciduria is expressed by the formation of L-cystine urinary stones which can provoke clinical manifestations.

According to our own statistical data from the analysis of 1,340 urinary stones, about 1.6% are composed of L-cystine [17]. During the last few years, systematic examinations have been undertaken in the district of Ioannina (Northwest Greece) in order to detect the incidence of cystinuria among the local population.

In the present study the diagnosis and treatment of cystinuric patients with stone formation from two cystinuric families is reported.

Materials and Methods

A screening procedure by means of the "Urocystin Test" in urine samples of 210 individuals was carried out (details see [18]). The diagnosis of cystinuria is relatively easy with the Urocystin Kit[1] which contains the following reagents:

1.	Nickel sulfate	28 mg
2.	Sodium hydrosulfite	4 mg
3.	Disodium-EDTA	25 mg
4.	Sodium dihydrogen phosphate	4 mg
5.	Sodium bicarbonate	50 mg
6.	Sucrose ad.	400 mg

When 4 ml of urine from a cystinuric patient are added to the "Urocystin Kit" a dark brown colouration develops within 3–4 min, due to the reduction of L-cystine to L-cysteine by the sodium hydrosulfite and the subsequent reaction of L-cysteine with the Nickel ions to form a brown coloured complex. The disodium EDTA controls the sensitivity of the reaction which turns positive if the concentration of L-cystine in the urine exceeds 50 mg/l. In normal urines with low L-cystine concentrations, below 50 mg/l, the presence of EDTA hinders the formation of the brown Nickel-cysteine complex. Therefore, the control urine shows a light blue colouration which can be easily distinguished from the brown colour developed in urines of cystinuric patients. (Caution must be taken in cases of administration of drugs containing sulfhydryl groups which may interfere with the Urocystin test and give erroneous positive results.)

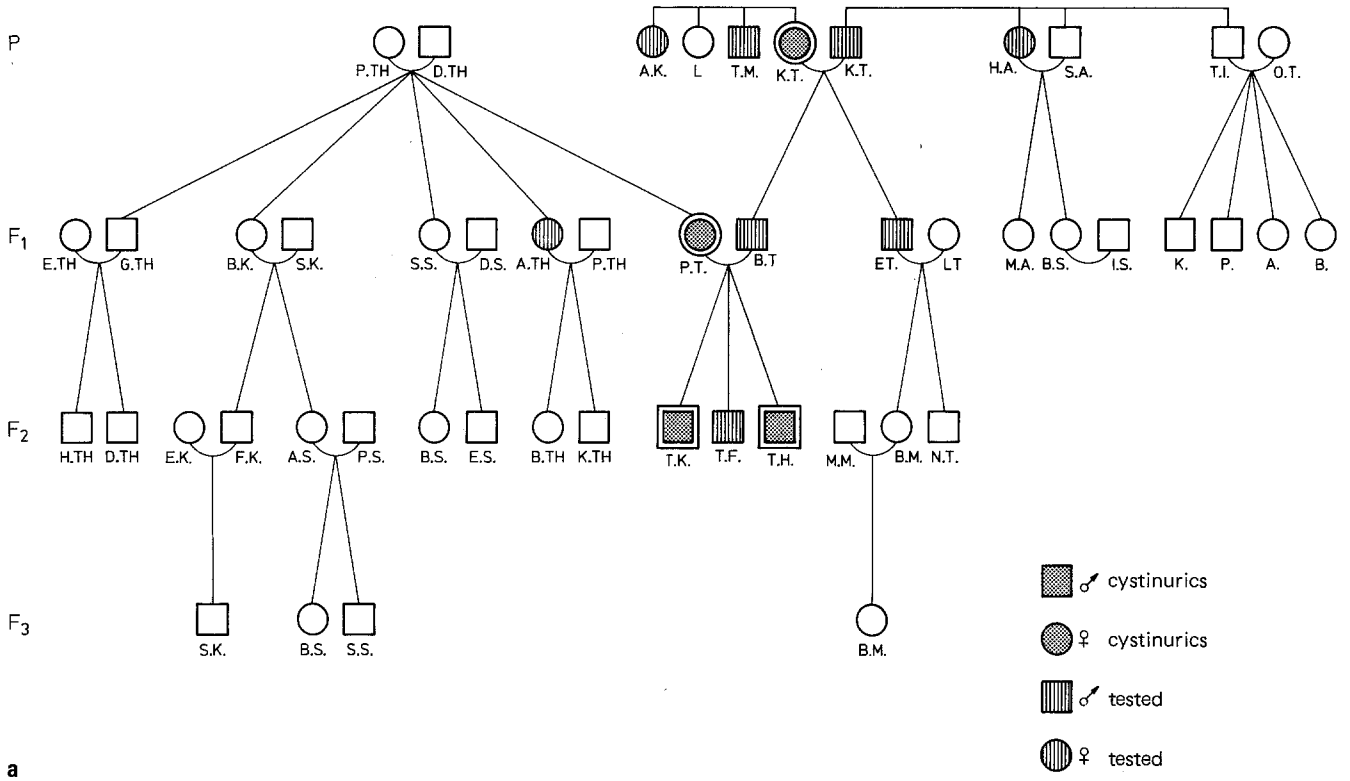
Supplementary examinations were carried out in urine sediments of cystinuric patients, in order to detect the characteristic hexagonal crystals of L-cystine.

Spontaneously eliminated L-cystine stones or those removed by surgery were analyzed by means of infra-red spectrography (Perkin Elmer Model 597, I. R. Apparatus).

Results

From the 210 urine samples investigated, 202 were negative and 8 were positive to "Urocystin Test". From the 8 positive cystinuric cases the sex distribution was 4 males and 4 females. Four of them were also L-cystine stone formers

[1] Urocystin Kit from Santen Pharmaceutical Co. Osaka, Japan



a

(three male and one female). From the remaining four positive cystinuric cases without stone formation, two were adult females and two were children, one three year old boy and a six year old girl; all four can be considered as asymptomatic cystinurics. The pedigrees of the eight cystinurics are demonstrated in Fig. 1. One of the three male stone formers (T. K.) has produced more than 100 stones which were spontaneously eliminated since 1974. Also during 1980 he was operated on twice (right kidney and bladder). His brother (T. H.), a cystinuric patient, was also operated on twice for urinary stones. The third patient (N. P.) underwent surgery in 1982 following an episode of acute renal failure.

In the present study, two family pedigrees, one from the village "Kato-Lapsista" and the other from "Marmara" were found to have offspring positive to the "Urocystin Test" with L-cystine concentration in urine above 75 mg/l.

The pedigree in "Kato-Lapsista" has 96 members coming from a progeny who married twice.

His family with his first wife has 63 members (35 ♂ and 28 ♀) including three cystinuric offspring aged 59, 50 and 6. However only 15 people were screened as the remainder of the family had left the region. His second family included 33 offspring (18 ♂ and 15 ♀). Among twelve screened members, a 3 years old boy was found positive for cystinuria. The pedigree in "Marmara" has 50 members (27 ♂ and 23 ♀). Four out of twelve tested were found positive for cystinuria. In this pedigree the female progeny and her daughter-in-law, as well as two second class male offspring are cystinurics. The L-cystine concentration in the urine tested revealed that the pedigree in "Cato-Lapsista" is a "completely recessive cystinuria" while the other in "Marmara" is an "incompletely recessive cystinuria".

All male L-cystine stone formers were treated with the drug α-mercaptopropionyl-glycine (MPG) or Thiola[2]. The drug Thiola is a sulfhydryl compound with a better dissolving capacity for L-cystine than D-penicillamine [15, 16] (Table 1).

Thiola was tested during the last decade clinically in numerous cases of urinary L-cystine stone formation with satisfactory results [1, 6, 7, 9, 10, 12, 20, 23, 24, 25, 26, 27, 31, 32, 33, 36]. Besides its prophylactic and L-cystine stone dissolving properties, Thiola was better tolerated than D-penicillamine and therefore the side effects caused by the drug during treatment were relatively limited. The L-cystine stone formers were treated initially with a dose of 300 mg daily, being increased to a maximum of 1,200 mg in divided dosage. When the urine became clear of L-cystine crystals, dosage was reduced to maintenance levels. No L-cystine stone recurrences have been noted since the administration of Thiola. The frequency of colic was also reduced and completely disappeared during the administration of Thiola. Thiola was generally well tolerated during the years of administration. Occasionally, some gastro-intestinal complains were reported and also a sulphurous smell of the urine and faeces. Proteinuria was also observed which disappeared when medication was stopped. Some cases of nephrotic syndrome during treatment with Thiola have

[2] Thiola from Santen Pharmaceutical Co. Osaka, Japan

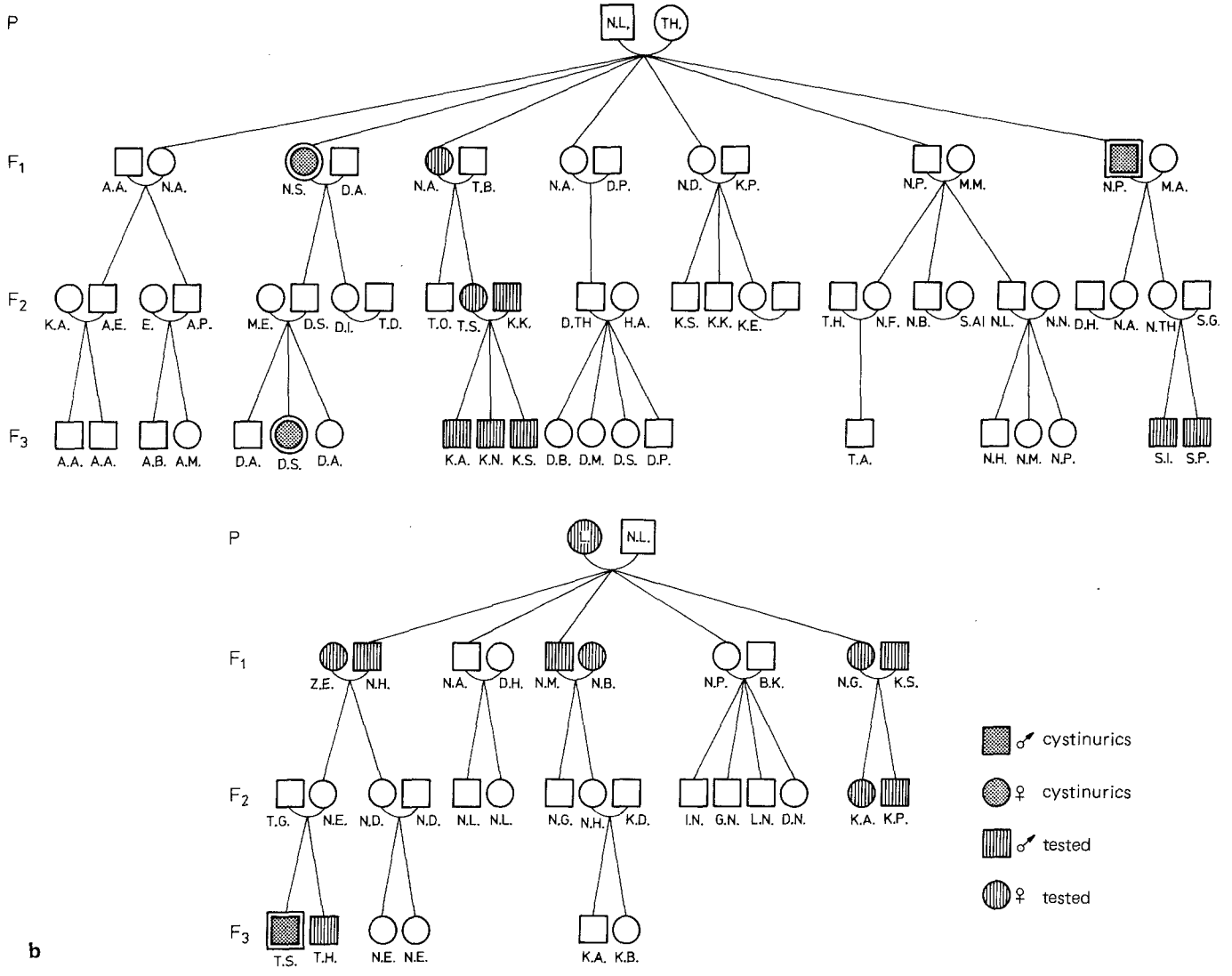


Fig. 1a. The pedigree in "Marmara" village. From the cystinuric female progeny (K. T.) a non-cystinuric male of the F_1 generation was married to the cystinuric P. T. Of the three sons of the F_2 generation, two were cystinurics (T. K. and T. H.). b The pedigrees in "Kato Lapsista" Village. The male progeny (N. L.) married twice. In the first generation two cystinurics, one male (N. P.) and one female (N. S.) were detected. In the second generation none; and in the third generation one cystinuric female (D. S.) who is the grandchild of the (N. S.) was diagnosed. From the second marriage of the progeny (N. L.) a cystinuric male was found in the third generation (T. S.)

been reported [28, 29]. Other side effects such as stomatitis, itching and gastric discomfort have been also observed [25]. Hales et al. [11] reported a case of myopathy due to mercapto-propionyl-glycine.

The clinical application of Thiola since 1970 including the newly discovered cases confirm the positive results of this drug for the prevention and treatment of the L-cystine urolithiasis.

Discussion

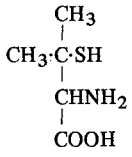
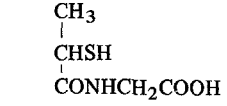
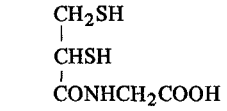
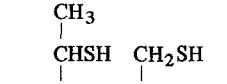
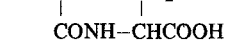
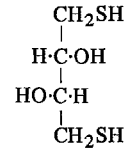
The "Urocystin Test" is a simple, rapid and reliable method which can be used as a "screening test" for the diagnosis of cystinuria. The positive cases can be confirmed by more specific diagnostic procedures such as microscopic detection of the characteristic hexagonal L-cystine crystals in the urinary sediment as well as with the infra-red spectrographic analysis of urinary stones from cystinuric patients.

As a routine method it has the additional advantage of being safer in laboratory work as compared with other methods using dangerous and poisonous compounds such as potassium cyanide for the reduction of L-cystine instead of the harmless sodium hydrosulfite.

Besides the 210 urine samples examined in Ioannina District, a further 738 people were screened before with the "Urocystin Kit" up to 1977, 948 cases altogether. Out of these, 37 cystinuric cases were diagnosed by means of the Kit.

Dent and co-workers [5] found that cystinuric patients have a defective renal absorption of L-cystine, with a cystine clearance of 100 ml/min compared to a normal of only 4 ml/min.

Table 1. Dissolution of 240.3 mg L-cystine (solid phase) with mono- and dithiols and their acute toxicity

Nr.	Chemical Formula	Substance	Amount of thiol		Dissolved L-cystine		Relative Molecular Activity	LD50 mg/kg	Animal	Application
			mg in 20 ml solution = Mol. W.	Concentration %	mg	%				
1	H_2O	Water			4.7	1.96				
2		D-penicillamine (β,β'-Dimethyl-cysteine)	185.65	0.93	59.3	24.60	100	2,000	rats	i.v.
3		Thiola (a-mercaptopropionyl-glycine)	163.20	0.82	92.4	38.40	156	2,000 1,400 4,190	mice mice mice	i.v. i.p. oral
4		N-(2,3-dimercaptopropionyl)-glycine	195.25	0.976	87.3	36.3	147	800	mice	i.p.
5		DL-a-mercapto-propionyl-DL-cysteine	209.16	1.05	118.2	49.2	200			
6		DL-a-mercapto propionyl-L-cysteine	209.16	1.05	141.2	58.8	240	2,092	mice	i.p.
7		Dithiothreitol	154.3	0.77	194.0	80.7	328	94 333	mice mice	i.v. s.c.

Cystinuric patients also have elevated renal clearance for arginine, lysine and ornithine. It is assumed that these aminoacids share a common renal transport mechanism while cystines reabsorption is unimpaired. On the other hand, further studies have revealed an abnormal intestinal absorption of the dibasic aminoacids.

In contrast to the results obtained with renal tissue, the uptake of lysine and arginine as well as of cystine was markedly impaired in the intestine of cystinuric patients. It was also found that cystinuric patients are unable to absorb cystine from the gut, while the intestinal absorption of cystine seems to be the same in cystinuria as in normal controls.

Genetically the disease is classified in two forms. The first designated "*completely recessive cystinuria*" concerns individuals of one family excreting either normal or grossly abnormal amounts of L-cystine in the urine. Intermediary amounts of L-cystine were not observed among the members of the completely recessive cystinuric families. In this form of cystinuria, active or mediated transport of L-cystine, lysine and arginine by the intestinal mucosa is totally abolished, a fact that is compatible with functional or structural absence of a specific enzymatic carrier protein.

In the second form of cystinuria designated "*incompletely recessive cystinuria*" the L-cystine excretion varied from normal to moderate and then up to high levels. In this form of cystinuria the intestinal transport of lysine and cystine varies in individuals of one family. However, the final genetic picture of cystinuria remains incomplete.

Dissolution of urinary L-cystine stones can be achieved either by alkalinization of the urine, because L-cystine is more soluble in alkaline solutions, or with specific litholytic drugs by means of a thiol – disulfide – exchange reaction where the less soluble L-cystine is transformed to the water soluble disulfide [2].

The first thiol derivative used clinically for the treatment of L-cystine lithiasis was D-penicillamine [3, 5, 8, 30, 35]. Other sulfhydryl compounds were found to be equally or even more effective than D-penicillamine [13, 14, 15]. Thiola was among the new sulfhydryl drugs and was found to be about 50% more effective than D-penicillamine in dissolving L-cystine stones [16, 34]. Further investigations revealed that by introducing a second HS-group to certain mono-mercapto compounds, the litholytic effect of these new dimercaptoderivatives could be enhanced [19]. The synthesis of new litholytic drugs possessing a better dissoving capacity for L-cystine and also less side effects will mark significant progress in the management of cystinuric disorders caused by the L-cystine lithiasis.

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