Childhood cystinuria in New South Wales

Results in children who were followed up after being detected by urinary screening in infancy

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SUMMARY Homozygous cystinuria was diagnosed in 45 children and 19 of their siblings in the course of routine urine screening of 6-week-old infants in New South Wales. These children were followed for up to 14 years. During this time there were 5 clinical episodes of renal disease which could be ascribed to cystinuria. There was normal mental development in all the children except one. Of 49 children over 3 years, 4 had height centiles less than the midparent height centile, while 45 had height centiles equal to or above the midparent centiles. Family testing in these 45 cases showed that 60% were type I cystinurics, and 35% were of the mixed or compound type (5% were not classified). Data from the parents and grandparents showed that renal tract calculi had occurred in 14 of them. This study shows that children with homozygous cystinuria, detected by urinary screening in infancy, rarely have renal symptoms. Mental development was normal as was growth in height. There was an increased incidence of noncystine stone formation among the relatives of these children. The incidence of homozygous cystinuria in New South Wales is one in 17 286.

Cystinuria is a disorder of renal tubular and intestinal transport of amino-acids, the main clinical association being renal calculi formation. Colliss *et al.* (1963) reported a height deficit in cystinurics compared with the general population, and Scriver *et al.* (1970) suggested that mental retardation was an associated feature. Many cases of cystinuric stone formers have been described, mainly in adults, although in the study of Bostrom and Hambræus (1964) in Sweden there were 9 children and adolescents, most of whom were identified by the clinical manifestations of calculus disease. We report the results in a group of children with homozygous cystinuria who were followed up after being identified by newborn screening of urine.

Methods

During a 13-year period (1964 to end of 1977), urine

samples from 950 767 6-week-old babies in New South Wales, 85% of the infant population, were examined by unidimensional paper chromatography (Wilcken *et al.*, 1973). If this first urine test showed markedly raised levels of cystine, lysine, or arginine (ornithine is not well separated by this method) a repeat test was requested. These first two tests were performed on urine-soaked blotting paper slips, but if the second test also showed a gross aminoaciduria consisting of cystine, lysine, or arginine, a liquid urine specimen was requested. A definitive diagnosis of homozygous cystinuria was made if this test showed high levels of cystine, lysine, and arginine, the baby usually being 6 months by this stage.

Children with homozygous cystinuria were seen for initial assessment and then at least yearly. At each visit they were assessed clinically, their general health was assessed, and their parents were also questioned specifically about the occurrence of back ache, abdominal pains, renal pain or colic, haematuria, frequency of micturition, and scalding of urine. They were asked about any medication the child had received, the diet, and details of fluid intake. Height and weight (without shoes) of the affected children

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were measured as were also the heights and weights of the siblings and parents seen at the follow-up. No correction was made for parent's age, all being under 40 years of age. The children's height centiles and the midparent height centiles were calculated using the tables of Tanner *et al.* (1970). The parents were encouraged to bring the whole family to each visit and unaffected siblings were also seen several times in most families.

Formal IQ testing in these patients was not routinely done. Achievement of milestones was recorded and parents were asked to rate the development of the child as slower than, the same as, or quicker than that of unaffected siblings. Children who had completed at least 2 years of schooling were assessed by their school reports and their class level for the age. If they were in the top class for the age grade, they were rated as good, if in the middle class as average, and if in the lowest class they were rated as slow. Their school level was also compared with their siblings.

The management of the children was the same in all cases, namely increased fluids to the limit of tolerance. None of the children had been given urinary alkalinisers, D-penicillamine treatment, or protein restriction at the time of this report. Four children (from 2 families) were investigated biochemically on a number of occasions, but have not been seen by us. Information was obtained from the family doctor by means of a specific questionnaire, as described above.

At each follow up, a sterile midstream specimen of urine was obtained and this was immediately set up for microurine analysis by standard methods. Urinary amino-acids were examined at each visit by high voltage electrophoresis, cystine was estimated quantitatively (Rootwelt, 1967), the presence of excess arginine confirmed by the Sakaguchi reaction (Smith, 1969), and cystine crystals were sought using the method of Hepler (1950). These examinations were performed on fasting first morning urine specimens in most patients but in some of the older ones on a 24-hour urine specimen. In 4 cases, urinary amino-acids were estimated on a Beckman Unichrome amino-acid analyser.

Once the patient had reached age 4 or 5, a plain x-ray of the abdomen was taken to exclude the presence of a calculus, and this was repeated 2 or 3 years later in some of the older patients. A detailed genetic history was also obtained at the initial visit with specific questioning about family history of renal calculi, consanguinity, and abortions, as well as about more general genetic conditions. Urine specimens from both parents and all siblings were tested in each family; in some families other family members were also tested.

Results

Of 950 767 babies tested between the start of screening in 1964 and the end of 1977, 45 were found to have cystinuria, 19 of 89 siblings tested were also affected, giving a total of 64 known cases (Table 1). 38 were boys and 26 girls, giving a sex ratio of 0.59 ($P > 0.2$). Ten of the 19 homozygous siblings were born after the proband and are shown as probandi themselves in the incidence figures and in the discussion of initial urinary test findings. In one family 2 affected siblings of the proband were unlike twins, and in another family with twins, one was homozygous and the other normal. The ages of the 64 children are shown in the Figure.

Initial urine testing showed a typical aminoaciduria

Table 1 Siblings of the cystinuric probandi

Probandi (n = 45)	Siblings			
	Homozygous	Hetero- zygous*	Normal	Total
Boys (n=28)	19-10	18-11	52-33	89-54
Girls (n=17)	- 9	- 7	-19	-35

*From 16 mixed or compound families.

Figure Age of 64 homozygous cystinuric patients. The scale for age ranges is not uniform, and changes in scale are shown by a space—for example the 12-14 block represents a 3-year period (1964-66), being years during which few cases were detected, and >1 block represents a one-year period (1977), a year in which there were many cases. The 7 patients, aged at least 15 years and born before 1964, were found when their families were tested. Two other siblings, older than the proband (one 10, one 11 years), had not been screened in infancy and were also detected by family testing.

at 6 weeks in 44 of 55 babies. In 11 patients, arginine was not detected in the first test, although the cystine and lysine levels were very high. Seven of these 11 patients showed the typical aminoaciduria in the second test; by this time the baby was usually 3 months. There were 4 boys in whom increased arginine excretion was not apparent at either of the first 2 blotting paper tests although cystine and lysine levels were very high; this was the indication for requesting a third test. Cystine, lysine, and arginine were all much increased in the subsequent liquid urine specimen in these children and have remained so.

Patients were definitively diagnosed at about 6 months, the 9 older siblings were diagnosed when family studies were undertaken. All were reviewed during the latter part of 1977 and first half of 1978. The age at last review ranged from 6 months to 22 years (oldest sibling) with mean age 7.6 years. 49 were at least 3 years and 28 were at least 8 years. Renal symptoms were present in 5 patients*. One boy had an episode of renal calculus formation at age 17½. One girl had episodes of right loin pain at 10½, and there were 2 cases of urinary tract infection, and a boy of 4 years had dysuria and a urinary tract infection; 4 months later a stone was detected on routine x-ray. There were also 3 children (2 boys, 1 girl) who were bed-wetters until about 9–10 years and there were 3 asymptomatic girls who showed urinary contamination, but not infection of urine, on routine microurine investigations at follow-up.

Routine x-rays of the abdomen had been performed in 42 of the children (in some of the older ones more than once) and in one case a calculus was seen which was asymptomatic. Routine tests for microscopic haematuria were negative in every patient at each follow-up.

The general health of the children was good and not significantly different from their siblings. There were 9 cases with noteworthy childhood conditions. One boy had a hemivertebra of L4 spine producing lumbar scoliosis; the adopted girl suffers from severe asthma requiring daily medication; one girl at 3½ years has grey carious irregular teeth; one boy aged 4 years had surgical correction of pyloric stenosis at 5 months; and one boy, free of renal symptoms, had a salivary gland calculus removed surgically at 7 years. Another boy aged 9 also had surgery for pyloric stenosis as a baby. One girl had mild minor retardation and attended the spastic centre until she was 5, and one boy had surgery for bilateral convergent strabismus. One further patient has petit mal epilepsy.

*Details of individual cases are available from authors on request.

The growth of these homozygous cystinuric children appeared to be within the normal range. The mean birthweight was 3.07 kg (boys and girls combined) with a range of 1.76 kg (in a 7-week preterm girl) to 4.28 kg. In 5 babies the birthweight was low due to prematurity (including the twins), there were 2 term babies in whom the birthweight was low, but in all the others it was average. Birth length was not as consistently recorded as birthweight and was not known for 20 of the children, but in the remainder the mean birth length was 52.5 cm. Two children had birth lengths <3rd centile, the rest were between 10th and 90th centiles.

Height of the children at last follow up (of the 49 patients over 3 years) showed that 28 were on the same centile as their midparent height centile, 18 were above, and 4 were below this. Three of these children had unaffected siblings who were also below the midparent height centile, and the fourth, a 4-year-old boy, was on the 10th centile for height, the midparent height being on the 50th centile, but he was an only child and so had no siblings for growth comparisons. The heights of the parents of the adopted girl were not known, but she was on the 50th centile for height.

Results of genetic studies are shown in Table 2 using the genetic types as described by Rosenberg *et al.* (1966). In one case there were no family data as the patient had been adopted. In one family, the results of family testing could not be reconciled with current concepts of the genetic inheritance of cystinuria. Of the remaining 43 families, 27 were type I and 16 were of mixed genetic type, 6 in which both parents were carriers and 10 with one normal parent and one heterozygous parent.

The sex ratio among the siblings of the patients can be seen in Table 1 and is 0.5 for the homozygous siblings, 0.61 for the heterozygous siblings, and 0.64 for the normal siblings. Although there appear to be slightly more boys, particularly among the normal siblings, this was not significant ($P > 0.1$). Data for sex ratio of newborn babies in N.S.W. were obtained from the Commonwealth Bureau of Statistics (Commonwealth Bureau of Statistics, 1978, personal communication).

There was no case of parental consanguinity in these families, nor was there any consanguinity

Table 2 Genetic types in 45 families

	No.	%
Type I	27	60
Type II-III	6	35.5
I-II/III	10	
Unknown*	1	2.25
Atypical†	1	2.25

*An adopted child, †Case 15.

known among either the maternal or paternal grandparents. One child was adopted, and this information was not known in this case. Among the siblings of the propositi, there were 13 early 1st trimester spontaneous abortions (in 9 of these families), 89 pregnancies proceeded to term. Given that 15% of all pregnancies abort, these findings indicate that there is no increased abortion rate among cystinuric heterozygotes. There were also 3 stillbirths and one case of premature labour of twins, neither of whom survived. Altogether, fetal loss in these families was not excessive.

Information about relatives with kidney stones was sought but it became clear during history taking that these data were inconsistent from family to family. Many relatives were reported to have had 'kidney trouble', but it was not always possible to verify the information. Only confirmed episodes of calculi among the parents and maternal or paternal grandparents are given, and hence this is a low estimate of the occurrence of renal calculi among relatives of the propositi. These data are shown in Table 3. These 14 relatives were tested and amino-acids were normal in 12 of them, where the calculi were clearly noncystine in composition. One paternal grandfather was a type II heterozygote cystinuric, and one maternal grandmother was a type III heterozygote. Stone analysis was not performed in either of these, but it would seem unlikely that the maternal grandmother had had cystine stones but possible that the paternal grandfather's stones contained cystine. In addition 2 of the fathers had each had a nephrectomy for congenital anomalies of the kidney.

Intellectual achievement of the patients was satisfactory. School progress is reported where at least 2 full years of schooling have been completed at last follow-up. This applied to 35 of the children, and of these, 14 were in the highest class for their age, 16 were in the middle for their age and considered average, and 5 were in the lowest class for their age. In this group of 5 slower children, one boy who was repeating his 3rd year of school was an only child,

2 boys had unaffected siblings considered to be average, one girl had 2 unaffected siblings also in the lowest class for their age, and one girl had an unaffected younger brother who had not yet started school. Of the 14 children in the highest class for their age, 3 were at the top and had excellent reports. One of these is the adopted girl, one girl was ahead of her two heterozygote siblings, and one boy, who had attended a special opportunity class for intelligent children in his area, was advanced compared with his homozygote brother and his 2 other unaffected siblings, who were all in the average class. Only 2 patients have completed their schooling, each was an elder sibling of a propositus. Both were average pupils at school, one is now an office worker and the other a student nurse.

The remaining 26 children include the babies, toddlers, preschool children and those who had not completed 2 full years of schooling. Developmental progress as assessed by milestones was entirely normal in 25 of these children. One child (Case 31) is mentally retarded in the mild to moderate range.

Discussion

Cystinuria is a disorder of the transport of cystine and the dibasic amino-acids in both the renal tubule and the intestine. The presence of markedly high levels of cystine, lysine, and arginine in the urine of our patients, often with cystine crystalluria, clearly classified them as homozygotes. No intestinal absorption studies were undertaken. It is not always possible in the initial tests to distinguish homozygotes from heterozygotes. In 4 of our patients the classical aminoaciduria was not found in the first two screening tests and appeared in the third and subsequent test. A definitive diagnosis in these children was delayed until their third test had been performed. Similar problems were encountered by Levy *et al.* (1972) who also found repeat testing necessary in some cases; their urine screening programme was the most comparable to our own. The programme in Wales (Bradley, 1975) was similar in structure, but cystine was detected by the nitroprusside spot test initially. We found the nitroprusside test to be very accurate for detecting cystine in the urine, but false-positives and false-negatives may occur (Smith, 1977).

The incidence of cystinuria, based on routine screening of urine from normal infants, has recently been reported by Thalhammer (1975) as part of a collaborative study from 6 centres; Levy reported an incidence of one in 15 841 in Massachusetts, Thalhammer one in 1100 in Vienna, Hyaneck in Prague found one in 22 065, Scriver in Montreal found one in 14 084, while in N.S.W. we reported an

Table 3 Relatives with renal calculi

Relative	No.
Mother	1
Father	—
Paternal	
grandmother	1
grandfather	3*
Maternal	
grandmother	4
grandfather	5*
	—
	14

*Two required nephrectomies; one was a heterozygote type II cystinuric, the propositus coming from a mixed family I-II/III.

incidence of one in 5069. On reviewing our data, it was apparent that some cases of type II heterozygotes were included. With these cases eliminated and using an extended population sample, the incidence in N.S.W. has been reduced to one in 17 286. This figure is very similar to the incidence reported by Levy, but is still quite different from those found by Thalhammer (one in 1100), Hyanek (one in 22 065), and Bradley (1975) (one in 27 059 cases). These various figures may reflect true differences in the incidence of homozygous cystinuria throughout the world, or they may be the result of other methods used to detect cases or different criteria for the diagnosis of homozygous cystinuria.

Colliss *et al.* (1963) reported on the stature of 44 patients with cystinuria, 42 of whom had had renal calculus disease. The mean height, adjusted to the adult male population, was found to be 2.5 cm less than that of controls. Our data indicate that homozygous cystinuric children are not smaller than normal children. The height of the cystinuric children was below that expected using the midparent height centiles (Tanner *et al.*, 1970) in only 4 children, but 3 of these were nevertheless on the same height centiles as their unaffected siblings. In one case the propositus was an only child. The parents' heights were generally measured when the patients were seen, in some cases the father's height was reported by the mother.

The birth length data presented here, although incomplete, suggest that affected children do not begin life shorter than normal children. It may be that the shorter stature noted by Colliss is the result of renal calculus disease, as most of her patients had renal calculi; however, our 2 patients with calculi, a 17-year-old and a 4-year-old boy, were both on the 75th centile for height.

Scriver *et al.* (1970) suggested that defective brain transport mechanisms in cystinuric patients may lead to mental retardation. No patient in our group could be considered to be mentally retarded due to cystinuria. One boy was retarded but, as he had other handicaps and congenital deafness, it would not seem justifiable to consider that the retardation was due to cystinuria. Excluding this patient, the intellectual achievement of 35 children who had completed at least 2 years of schooling showed a wide scatter with only 5 being in the lowest class for their ages; they were, however, certainly not retarded. Of the remaining children, the milestones were all adequate and comparable with unaffected siblings, although 10 children born in 1977 were still too young to assess this accurately. Formal psychometry was not performed. Gold *et al.* (1977) did use psychometry on 26 patients in Canada and the intelligence levels of their patients were no

different from those of their unaffected siblings or the normal population.

If mental retardation affects 1.5% of the population and the incidence of homozygous cystinuria is one in 15 000, then the chance of both conditions occurring coincidentally would be one in 1 000 000, or 5 people in N.S.W. As noted, only one such child was found.

The general health of these children was good and was in no way impaired by also having homozygous cystinuria. A possible exception is the finding of 2 cases of pyloric stenosis, but further studies will be required to prove if an association exists between the two conditions. Considering the number of patients and the length of time of follow up, there were very few renal symptoms. There were only 2 proved episodes of calculus formation, one episode of renal pain for which no cause was found, and 2 cases of urinary tract infection. This study suggests that renal symptoms in children with cystinuria are rare. The management of all these children consisted of increased fluid intake to the limit of tolerance. It would be satisfying to think that follow-up of the patients and encouragement to maintain increased fluids was the reason for lack of renal symptoms but, in fact, it was difficult to know in most cases what the child's fluid intake actually was, and fluid intake appeared to fluctuate from time to time. Without a control group and stringent control of fluid intake, this aspect of management cannot be adequately assessed.

Among this group of homozygote cystinurics, 60% of the informative cases were of type I inheritance, and 35% were mixed or compound types (Table 2). Bostrom and Hambræus (1964), in their extensive survey of cystinuria in Sweden, showed that of 59 families studied, 10 contained demonstrable heterozygotes (which they called semicystinuria), giving 83% type I families. Out of 21 families studied by Harris and Warren (1953), 6 contained demonstrable heterozygotes, leaving 71% type I cystinurics (which they named completely recessive); Rosenberg *et al.* (1966) studied 15 patients and found 60% to be type I. The frequency of the different genetic types of cystinuria appears to vary with different populations, although in every large study, the completely recessive or type I form is the most common. This applies to all those studies where the propiti have been mainly stone-forming patients, and also to our study where the patients were detected in an unselected way by urinary screening. It appears, therefore, that the genetic type of the patient is not an important factor for cystine stone formation in a patient with homozygous cystinuria.

We found 14 relatives who had had renal calculi among 270 parents and grandparents (Table 3);

accurate figures for the incidence of renal calculi in this population are not available, but using the annual incidence of Malek (1977) of one in 1000 of the population in the USA, it does appear that our findings show an increase of noncystine stone formation among the relatives of cystinurics.

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References

- Bostrom, H., and Hambræus, L. (1964). Cystinuria in Sweden. VII. Clinical, histo-pathological, and medico-social aspects of the disease. *Acta medica Scandinavica*, **175**, Supplement 411.
- Bradley, D. M. (1975). Screening for inherited metabolic disease in Wales using urine-impregnated filter paper. *Archives of Disease in Childhood*, **50**, 264-268.
- Colliss, J. E., Levi, A. J., and Milne, M. D. (1963). Stature and nutrition in cystinuria and Hartnup disease. *British Medical Journal*, **1**, 590-592.
- Gold, R. J. M., Dobrinski, M. J., and Gold, D. P. (1977). Cystinuria and mental deficiency. *Clinical Genetics*, **12**, 329-332.
- Harris, H., and Warren, F. L. (1953). Quantitative studies on the urinary cystine in patients with cystine stone formation and in their relatives. *Annals of Eugenics*, **18**, 125-171.
- Hepler, O. I. (1950). *Manual of Clinical Laboratory Methods*, fourth edition, p. 21. Thomas: Springfield.
- Levy, H. L., Madigan, P. M., and Shih, V. E. (1972). Massachusetts metabolic disorders screening program. I. Technics and results of urine screening. *Pediatrics*, **49**, 825-836.
- Malek, K. S. (1977). Renal lithiasis: a practical approach. Review article. *Journal of Urology*, **118**, 893-901.
- Rootwelt, K. (1967). Quantitative determination of thiols and disulphides in urine by means of Ellman's reagent and thiolated sephadex, and its application in cystinuria. *Scandinavian Journal of Clinical and Laboratory Investigation*, **19**, 325-330.
- Rosenberg, L. E., Downing, S., Durant, J. L., and Segal, S. (1966). Cystinuria: biochemical evidence for three genetically distinct diseases. *Journal of Clinical Investigation*, **45**, 365-371.
- Scriver, C. R., Whelan, D. T., Clow, C. L., and Dallaire, L. (1970). Cystinuria: increased prevalence in patients with mental disease. *New England Journal of Medicine*, **283**, 783-786.
- Smith, A. (1977). Evaluation of the nitro-prusside test in the diagnosis of cystinuria. *Medical Journal of Australia*, **2**, 153-155.
- Smith, I. (1969). *Chromatographic and Electrophoretic Techniques*, third edition, p. 121. Heinemann: London.
- Tanner, J. M., Goldstein, H., and Whitehouse, R. H. (1970). Standards for children's height at ages 2-9 years allowing for height of parents. *Archives of Disease in Childhood*, **45**, 755-762.
- Thalhammer, O. (1975). Frequency of inborn errors of metabolism, especially PKU, in some representative newborn screening centres around the world. A collaborative study. *Humangenetik*, **30**, 273-286.
- Wilcken, B., Smith, A., Gaha, T. J., McLeay, A. C., and Brown, D. A. (1973). Screening for metabolic diseases in New South Wales. *Medical Journal of Australia*, **1**, 1129-1133.

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