Cystinuria: a new genetic variant

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Summary
1. A family is reported with an unusual type of cystinuria.
2. The propositus presented with a cystine renal stone; the renal tubular reabsorption of cystine was grossly abnormal but the tubular reabsorption of ornithine, lysine and arginine was only slightly less than normal.
3. One of the children of the propositus excreted cystine and lysine in increased amounts typical of type II heterozygotes for cystinuria.
4. The renal transport defect in this family may represent one end of the spectrum of cystinuria or it may be a form akin to isolated hypercystinuria.

Key words: amino acids, cystinuria, renal stones.

Introduction
Cystine was first isolated in 1810 from a bladder stone and Garrod (1908) described the disorder as an 'inborn error of metabolism'. However, it was not until the application of microbiological assay of amino acids (Yeh, Frankl, Dunn, Parker, Hughes & Gyorgy, 1947) and ion-exchange chromatography (Stein, 1951) that ornithine, lysine and arginine were also shown to be excreted in excess in cystinuria. The finding of normal plasma concentrations of these amino acids suggested that the urinary losses were due to a defect in the transport of these amino acids by the renal tubule (Dent & Rose, 1951). The defect affects all four amino acids in homozygotes whereas heterozygotes may either excrete increased amounts of cystine and lysine or may have a normal urinary amino acid output (Harris, Mittwoch, Robson & Warren, 1955). Oral loading studies demonstrated that a transport defect also affected the intestinal absorption of these amino acids (Milne, Asatoor, Edwards & Loughridge, 1961). Since then much work has been carried out on cystinuria and in particular the underlying transport defect (see review by Scriver & Rosenberg, 1973). However, in the last few years evidence has accumulated to suggest that the transport of cystine can be independent of the transport of the basic amino acids. Isolated lysinuria (Kekomaki, Visakorpi, Perheentupa & Saxen, 1967; Whelan & Scriver, 1968) and isolated cystinuria (Brodehl, Gellissen & Kowalewski, 1967) have been reported.

Case report
Patient B.G. is a woman of 57 years who was recently reinvestigated as part of a review of patients with both ileostomy and urinary stone formation, being carried out at another hospital. She was referred to us when a urinary nitroprusside test was shown to be positive. She had been quite well until the age of 33 years, when she began having bouts of diarrhoea and abdominal pain. At the age of 38 years she had a severe attack and diagnosis of ulcerative colitis was made; an ileostomy was raised and she underwent total colectomy. For the last 20 years, the ileostomy has functioned well, giving trouble only on rare occasions. At the age of 43 years she had an attack of low back pain, radiating to her left buttock; this was attributed to a prolapsed intervertebral disc but she was also shown to have a stone in her left kidney and a
Proteus urinary tract infection. She had no further trouble for 3 years, at which time she developed left renal colic and a stone was removed by nephrolithotomy. Qualitative chemical analysis of the stone showed it to contain cystine. Her urinary cystine estimated by polarography was 2.9 mmol/l and she was therefore considered to have cystinuria. Urinary amino acid chromatography was not carried out at this time. She was encouraged to maintain a high fluid intake but did not manage to drink more than 5 pints (2.5 l) each day. She was given a low methionine diet but found this too difficult to maintain. Since removal of her stone 11 years ago, she has passed no stones, has had no evidence of urinary tract infection and plain abdominal X-ray at the present time is normal. She is now quite well apart from the recent onset of rheumatoid arthritis in her hands. There is no family history of kidney disease or renal stones. Her mother died of pulmonary tuberculosis (aged 37 years); her father died of chronic bronchitis (aged 71 years), and she is an only child. Her three children and six grandchildren are alive and well.

Materials and methods

Specimens

Blood. Samples for amino acid analysis were obtained between 09.00 and 10.00 hours after an overnight fast. Blood was treated with heparin and the plasma separated at 4°C. Norleucine was added as an internal standard and the plasma proteins were precipitated with salicylsulphonic acid within 15 min of the blood being taken. The deproteinized plasma was then stored at −20°C before analysis.

Blood was obtained at the same time for the analysis of urea, calcium, phosphate, alkaline phosphatase and routine liver-function tests. These were carried out by routine methods (Technicon).

Urine. Urine was collected into hydrochloric acid (20 ml of 6 mol/l HCl/24 h sample), and stored at −20°C before analysis of amino acids, creatinine, calcium and phosphate.

Amino acid analysis

This was carried out on a Technicon NC-1 amino acid analyser with a 0.64 cm x 150 cm column of Chromobeads type B. Gradient elution was carried out over 16 h with sodium citrate buffers (Purdie, Gravelle & Hanafi, 1968); temperature programming was incorporated to ensure adequate separation of the methylhistidines from lysine (Purkiss, 1967).

Results

The urinary amino acid excretion of the family G is shown in Tables 1 and 2. Table 1 includes all members of the family that have been tested and, since some analyses could only be carried out on random urine specimens, all the results are expressed in terms of creatinine excretion. For comparison purposes, the members of the family excreting normal amounts of cystine and the dibasic amino acids are marked. Table 2 shows the 24 h amino acid excretion of those members of the family from whom it was possible to obtain 24 h urine samples; these results have been corrected to the standard surface area of 1.73 m².

The major abnormality of the patient (B.G.) was the increased cystine excretion (twenty-seven times that of the normal mean value). Ornithine, lysine and arginine were increased to a lesser extent; all other amino acids were normal. However, in R.G. the major abnormality was a fourteen-fold increase
Cystinuria

**Table 1. Urinary amino acid excretion of family G**
Molecular weights of the amino acids are given in parentheses. Cystine was calculated as cystine, not \( \frac{1}{2} \)-cystine.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Glycine (75:1)</th>
<th>Cystine (240:3)</th>
<th>Ornithine (132:2)</th>
<th>Lysine (146:2)</th>
<th>Arginine (174:2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.G.</td>
<td>720</td>
<td>1980</td>
<td>177</td>
<td>755</td>
<td>80</td>
</tr>
<tr>
<td>G.G.(^{(1)})</td>
<td>605</td>
<td>37</td>
<td>24</td>
<td>66</td>
<td>10</td>
</tr>
<tr>
<td>H.G.(^{(1)})</td>
<td>2425</td>
<td>67</td>
<td>24</td>
<td>104</td>
<td>19</td>
</tr>
<tr>
<td>D.G.(^{(1)})</td>
<td>1733</td>
<td>96</td>
<td>47</td>
<td>104</td>
<td>33</td>
</tr>
<tr>
<td>R.G.</td>
<td>945</td>
<td>363</td>
<td>124</td>
<td>1965</td>
<td>65</td>
</tr>
<tr>
<td>L.G.</td>
<td>1900</td>
<td>148</td>
<td>64</td>
<td>864</td>
<td>58</td>
</tr>
<tr>
<td>K.G.(^{(1)})</td>
<td>813</td>
<td>83</td>
<td>59</td>
<td>136</td>
<td>14</td>
</tr>
<tr>
<td>W.G.(^{(1)})</td>
<td>2213</td>
<td>41</td>
<td>95</td>
<td>296</td>
<td>28</td>
</tr>
<tr>
<td>P.G.(^{(1)})</td>
<td>2027</td>
<td>83</td>
<td>90</td>
<td>197</td>
<td>28</td>
</tr>
<tr>
<td>B.A.G.(^{(1)})</td>
<td>1267</td>
<td>37</td>
<td>6</td>
<td>44</td>
<td>10</td>
</tr>
</tbody>
</table>

\(^{(1)}\) Excretion considered normal.

**Table 2. Urinary amino acid excretion in subjects B.G., R.G., D.G. and L.G.**

<table>
<thead>
<tr>
<th></th>
<th>B.G.</th>
<th>R.G.</th>
<th>D.G.</th>
<th>L.G.</th>
<th>Control (mean±sd, ( n = 6 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total volume (ml/24 h)</strong></td>
<td>1270</td>
<td>1300</td>
<td>1670</td>
<td>1470</td>
<td></td>
</tr>
<tr>
<td>Cystine</td>
<td>1540</td>
<td>1828</td>
<td>1976</td>
<td>1470</td>
<td>705±460</td>
</tr>
<tr>
<td>Ornithine</td>
<td>270</td>
<td>157</td>
<td>169</td>
<td>73</td>
<td>220±169</td>
</tr>
<tr>
<td>Lysine</td>
<td>557</td>
<td>300</td>
<td>752</td>
<td>657</td>
<td>3700±2560</td>
</tr>
<tr>
<td>Arginine</td>
<td>145</td>
<td>72</td>
<td>77</td>
<td>58</td>
<td>131±77</td>
</tr>
<tr>
<td>Glycine</td>
<td>637</td>
<td>746</td>
<td>840</td>
<td>584</td>
<td>2750±1560</td>
</tr>
</tbody>
</table>

\(^{(1)}\) Range.

in mean lysine excretion above the normal mean value, whereas cystine, ornithine and arginine were increased only nine-, five- and three-fold respectively. Both B.G. and R.G. had normal plasma amino acid concentrations. In L.G., the cystine and lysine excretion was increased when related to creatinine excretion but apparently normal when related to surface area, although these results were calculated from the same sample. Although for D.G. the absolute excretion of cystine, ornithine and arginine was increased, that of glycine was proportionately increased, suggesting that this was more than a 24 h collection. This is supported by the fact that all these figures become normal when related to creatinine excretion. Unfortunately further specimens could not be obtained. All other members of the family that were investigated had normal urinary and plasma amino acid concentrations.

The renal clearance of amino acids was calculated for the patient (B.G.) and her son (R.G.) from determination on the fasting plasma and the 24 h urinary excretion. These results, corrected to a standard surface area, are shown in Table 3.

The renal clearance data in Table 3 confirm the massive renal leak of cystine in the patient (B.G.). For R.G., increased clearance of cystine and lysine was observed.

To assess renal tubular reabsorption of amino acids the amino acid clearances \( (C_r) \) were related to the creatinine clearance \( (C_{cr}) \) by the following formula:

\[
\text{Tubular reabsorption (\%)} = 100 - \left( \frac{C_r}{C_{cr}} \right) \times 100
\]
TABLE 3. Clearances of amino acids and creatinine

<table>
<thead>
<tr>
<th>Clearances (ml min⁻¹ 1.73 m⁻²)</th>
<th>Normal (mean ± SD, n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B.G.</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.19</td>
</tr>
<tr>
<td>Cystine</td>
<td>29.2</td>
</tr>
<tr>
<td>Ornithine</td>
<td>1.82</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.35</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.71</td>
</tr>
<tr>
<td>Creatinine</td>
<td>54</td>
</tr>
</tbody>
</table>

Surface area (m²) 1.70 1.90 1.73

(1) Not calculated because of technical difficulties.

These values are shown in Table 4 and confirm a major defect in cystine reabsorption in B.G. A small reduction in the reabsorption of the other dibasic amino acids in B.G. was also found. For R.G. cystine and lysine reabsorption was slightly impaired.

Creatinine clearance measurements were carried out on B.G. and R.G. and are also shown in Table 3. Routine liver-function tests (serum bilirubin, alkaline phosphatase, albumin, globulin, aspartate transaminase and 2-hydroxybutyrate dehydrogenase) were carried out in B.G. and R.G. and the results were within normal limits.

In view of the association with familial hypoparathyroidism in the previously reported cases of isolated cystinuria (Brodehl et al., 1967), the serum concentration and urinary excretion of calcium and phosphate were determined for B.G., R.G. and D.G., and the urinary excretion only for L.G. All these values were within normal limits.

Discussion

In classical cystinuria, the clinical and biochemical findings are extremely variable (Crawhall, Scowen, Thompson & Watts, 1967). Usually patients excrete grossly elevated amounts of cystine, ornithine, lysine and arginine in their urine. They suffer from recurrent cystine stones, which form because of the low solubility of cystine in urine. This variability manifests itself in a number of ways, such as the age at clinical presentation (2–60 years), the frequency of stone formation and, biochemically, as the absolute and relative amino acid excretion rates.

TABLE 4. Renal tubular reabsorption of amino acids in subjects B.G. and R.G.

See the text for calculation of percentage tubular reabsorption.

Renal tubular reabsorption (%)

<table>
<thead>
<tr>
<th>Cystinuria(1)</th>
<th>B.G.</th>
<th>R.G.</th>
<th>Homozygotes</th>
<th>Heterozygotes type II</th>
<th>Normal(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystine</td>
<td>45.2</td>
<td>52.6</td>
<td>47.6</td>
<td>96.9</td>
<td>96.3 (87.7–99.3)</td>
</tr>
<tr>
<td>Ornithine</td>
<td>96.6</td>
<td>96.0</td>
<td>96.9</td>
<td>99.8</td>
<td>70.9 (57.6–77.7)</td>
</tr>
<tr>
<td>Lysine</td>
<td>97.5</td>
<td>95.7</td>
<td>96.9</td>
<td>93.2</td>
<td>58.7 (26.0–69.8)</td>
</tr>
<tr>
<td>Arginine</td>
<td>98.7</td>
<td>99.1</td>
<td>—(2)</td>
<td>99.6</td>
<td>37.5 (25.9–68.4)</td>
</tr>
</tbody>
</table>

(1) Mean values and range (Morin, Thompson, Jackson & Sass-Kortsak, 1971).
(2) Tubular secretion in many cases.
(3) Not calculated because of technical difficulties.
The present case may reflect this variability or it may be considered as hypercystinuria (Brodehl et al., 1967). Alternatively it may be a separate abnormality of cystine transport, between these two extremes.

The amino acid findings in the propositus do not differ significantly from those reported by Brodehl et al. (1967) in their study of two children, but the calcium and phosphate excretions in our propositus and her family were normal. The renal tubular reabsorption of cystine was more impaired in this subject (48%) than in the children previously reported by Brodehl (75-78%). However, some differences in the renal transport of the basic amino acids have been shown. In the original cases, the tubular reabsorption of ornithine, lysine and arginine was indistinguishable from that of normal control subjects, whereas in the propositus the tubular reabsorption was in the lower range of type II heterozygotes for cystinuria. Morin, Thompson, Jackson & Sass-Kortsak (1971) found no difference in the renal handling of basic amino acids between normal subjects and type I heterozygotes and considerable overlap between these groups and type II heterozygotes.

It is possible that this case represents an extreme example of a type II heterozygote. A linear relationship between cystine and lysine excretion in cystinuric families has been demonstrated (Harris et al., 1955). In two of the subjects (Cy 14 and Cy 20), of that study, probands (stone-formers) had cystine excretion either in the lower homozygote range or upper heterozygote range, and lysine and arginine excretion fell in the mid-heterozygote range. But, in both cases, the relation between cystine and lysine excretion was not significantly abnormal (see Table 1, Harris et al., 1955), whereas, for the present patient (B.G.), the ratio is considerably removed from their data. For R.G. the ratio falls in their heterozygote range. Crawhall, Saunders & Thompson (1966), using a similar method of representation, also observed a linear relation between cystine and lysine excretion. Again B.G. diverges significantly from their results whereas R.G. falls within their range. L.G. has been classified as a heterozygote in Fig. 1, because of the raised cystine and lysine excretion when related to creatinine in the family table (Table 1), but her apparent normality when the values are related to surface area (Table 2) emphasizes the difficulties when working with children.

Therefore, when the relation of cystine to basic amino acid excretion is considered, B.G. differs considerably from these sets of data on cystinuric patients and their relatives. Although dibasic amino acid clearances were raised, because of the much higher cystine clearance the condition of B.G. would appear more like hypercystinuria than cystinuria. It is feasible that a raised excretion of cystine could cause some slight increase in dibasic amino acid excretion by competitive inhibition of overlapping renal transport processes. Lysine infusion had been shown to increase cystine excretion (Lester & Cusworth, 1973) but owing to the low solubility of cystine it has not been possible to determine experimentally if the reverse effect can occur. This mechanism could explain the slight reduction in tubular reabsorption of the other dibasic amino acids found in B.G.

Many recent studies have demonstrated cystine transport, or at least one mode of cystine transport, to be independent of dibasic amino acid transport. Mutual competition for transport between the dibasic amino acids but not between cystine and basic amino acids has been demonstrated in rat kidney cortex (Rosenberg, Downing & Segal, 1962). This finding was extended to human kidney (Fox, Thier, Rosenberg, Kiser & Segal, 1964). These findings suggest that, as well as hypercystinuria, the related disorder of renal loss of basic amino acids alone could also occur. Kekomaki et al. (1967) reported a trait of familial protein intolerance with deficient transport of basic amino acids alone. Since then a member of a French Canadian family with no major clinical symptoms has been reported (Whelan & Scriver, 1968) to excrete excess of basic amino acids. Two other recent reports (Oyanagi, Miura & Yamanouchi, 1970; Brown, Fabe, Farall & Adams, 1972) have subjects with associated lysinuria and mental retardation. By a combination of gut transport studies in vitro and oral loading tests, Rosenberg, Downing, Durant & Segal (1966) presented evidence for three genetically distinct forms of cystinuria. In their type II group there is no intestinal uptake of lysine and only slight uptake of cystine, whereas in type I subjects cystine and lysine uptake is totally absent, and in type III uptake of both is present but slightly less than that of normal subjects. In one case of hyperdibasicaciduria, normal intestinal transport of cystine occurred in the presence of impaired lysine absorption (Whelan & Scriver, 1968). It has not been possible to perform any intestinal
transport studies in the present case, so that it is not known whether an intestinal defect was present or not.

The renal transport findings appeared to differentiate subject B.G. from classical cystinuria, but this conclusion was cast in doubt by the finding that one of the patient's children had a urinary excretion pattern and renal clearance data characteristic of a type II heterozygote for cystinuria.

It appears that in homozygote cystinuria the magnitude of the transport defect varies widely among the different amino acids as well as among the different patients. Crawhall et al. (1967) concluded that this extreme range could be due to either separate amino acid carriers or the effect of a number of genes on one common carrier. It may be that hypercystinuria represents one branch of this wide genetic variation. If hypercystinuria is a separate entity from cystinuria, the finding of a urinary amino acid pattern typical of cystinuria in R.G. is difficult to explain. The possibility that the father (G.G.) was a type I (i.e. completely recessive) heterozygote for cystinuria cannot be excluded and the possible inheritance of both hypercystinuria and cystinuria in R.G. produced a urine pattern typical of a type II heterozygote.

It is possible that this patient's ileostomy was a contributing cause in her stone formation, since an increased incidence of stone formation has been reported in such patients, but in these cases the stones usually contain calcium or uric acid (Deren, Porush, Levitt & Khilnani, 1962; Ritchie, 1971; Bennett & Hughes, 1972). In this case the stone was composed of cystine and we therefore consider that, apart from possible dehydration, the ileostomy contributed little if anything to the stone formation. If such a patient had recurrent cystine stones, treatment with a high fluid intake or D-penicillamine could be considered.

Acknowledgments

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