

## LONG-TERM TREATMENT OF GIRLS WITH ORNITHINE TRANSCARBAMYLASE DEFICIENCY

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**ABSTRACT**

**Background** Ornithine transcarbamylase is an X-linked mitochondrial enzyme that catalyzes the synthesis of citrulline from carbamoyl phosphate and ornithine. A deficiency of this enzyme leads to hyperammonemia and hyperglutaminemia. In boys the disease is often fatal when its onset occurs during the neonatal period, but it is milder when onset occurs later in childhood. Heterozygous girls may be normal or may have episodes of hyperammonemic encephalopathy and decline in cognitive function. We report here on the long-term outcome in girls with ornithine transcarbamylase deficiency enrolled in studies of treatments designed to activate new pathways of waste-nitrogen excretion.

**Methods** We studied 32 girls (age, 1 to 17 years) with ornithine transcarbamylase deficiency who had had at least one episode of encephalopathy. The patients were assigned to treatment that consisted of sodium benzoate, alone or in combination with sodium phenylacetate or sodium phenylbutyrate, or sodium phenylbutyrate alone. Collaborating physicians provided clinical, metabolic, and developmental data at specified intervals.

**Results** Patients treated according to these protocols had greater than 90 percent survival at five years and maintained appropriate weight for height. The frequency of hyperammonemic episodes decreased with increasing age and with sodium phenylacetate or sodium phenylbutyrate treatment. Although the mean IQ before treatment was in the low average range, 19 of the 23 girls in whom intelligence was tested longitudinally had stable test scores.

**Conclusions** Girls with symptomatic ornithine transcarbamylase deficiency who are treated with drugs that activate new pathways of waste-nitrogen excretion have fewer hyperammonemic episodes and a reduced risk of further cognitive decline. (N Engl J Med 1996;335:855-9.)

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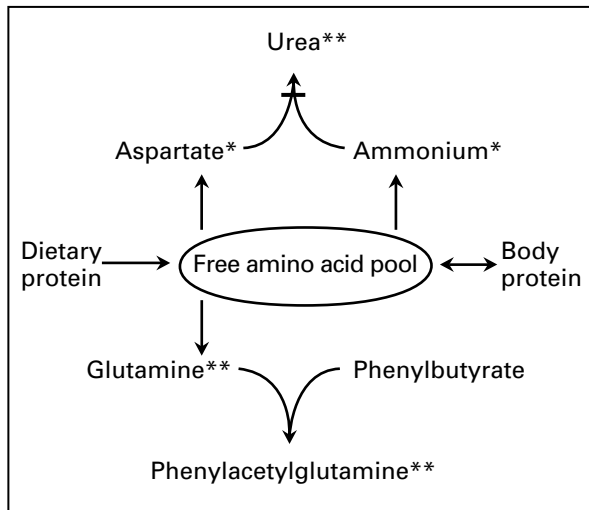
**O**RNITHINE transcarbamylase (ornithine carbamoyltransferase) deficiency is an X-linked disorder in which the synthesis of citrulline, and hence urea, from carbamoyl phosphate and ornithine is impaired. The consequences include hyperammonemia, hyperglutaminemia, hypoargininemia, hypocitrullinemia, and episodic encephalopathy that, if uncontrolled, results in brain injury and death. The most dramatic form of the disease occurs in newborn boys as cata-

strophic hyperammonemic encephalopathy; hemizygous males who survive the neonatal period have a poor neurologic outcome, with a high incidence of mental retardation, cerebral palsy, and seizures.<sup>1</sup> It is now apparent, however, that the disease may occur in males from the neonatal period through adulthood,<sup>2</sup> with late-onset disease associated with a milder phenotype.<sup>3</sup> This variable phenotypic expression in males is presumably a result of different mutant alleles, some of which permit residual ornithine transcarbamylase activity.

Clinical manifestations in females carrying a mutation at the ornithine transcarbamylase locus range from apparent normality to profound neurologic impairment.<sup>4</sup> Thirteen symptomatic girls ranging in age from 6 months to 14 years at the time of diagnosis all had a history of irritability, vomiting, and lethargy, and most avoided dietary protein.<sup>5</sup> Ataxia, progressive encephalopathy, developmental delay, and delayed physical growth each occurred in at least five of the girls, and three had seizures. Five of the 13 girls had IQs of less than 70 at the time of diagnosis, and IQ testing several months later indicated that another 4 had borderline mental retardation (IQ, 70 to 84). Variable expression in females is most likely a function of the proportion of hepatocytes in which the active X chromosome bears the mutant allele, although different mutant alleles may also have varying effects.

Therapy for this disease is directed at decreasing the requirement for urea biosynthesis by decreasing dietary nitrogen intake and by increasing waste-nitrogen excretion (Fig. 1). The latter may best be done by prescribing sodium phenylbutyrate, a precursor of sodium phenylacetate that conjugates with glutamine to yield phenylacetylglutamine, a waste-nitrogen compound that is rapidly excreted in the urine.<sup>6</sup> For patients treated with phenylbutyrate who have residual ornithine transcarbamylase activity, nitrogen is diverted from the urea cycle and endogenous urea synthesis is suppressed. However, the urea cycle remains functional and may be reactivated to maintain nitrogen homeostasis.<sup>6</sup> We report here on

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**Figure 1.** Biochemistry of Ornithine Transcarbamylase Deficiency and Its Treatment.

Ornithine transcarbamylase and other urea-cycle enzymes catalyze the incorporation of nitrogen not used for net synthetic purposes into urea; aspartate and ammonium are the nitrogen donors. When urea synthesis is impaired by ornithine transcarbamylase deficiency (blocked arrow), the administration of sodium phenylbutyrate activates the biosynthesis of phenylacetylglutamine, which replaces urea as a waste-nitrogen product; glutamine is the nitrogen donor. The asterisks denote the number of nitrogen atoms.

the long-term outcome in 32 girls with symptomatic ornithine transcarbamylase deficiency who were enrolled in therapeutic trials of sodium phenylacetate and related compounds before 1991.

## METHODS

### Enrollment

From 1979 through 1990, 59 girls less than 18 years of age were referred to the Johns Hopkins Hospital for the evaluation of symptomatic ornithine transcarbamylase deficiency. The diagnosis of ornithine transcarbamylase deficiency was based on orotic aciduria during hyperammonemia and a normal plasma amino acid profile except for increased glutamine and alanine concentrations and decreased citrulline and arginine concentrations. All the patients had had at least one episode of encephalopathy; hepatic ornithine transcarbamylase activity, as measured in 16 patients, ranged from undetectable to 20 percent of normal.

A total of 39 girls were enrolled in a series of protocols approved by the Food and Drug Administration (FDA) and the institutional review boards of the hospitals where they received treatment; informed consent was obtained from all the patients or their parents. Thirty-three girls were white, one was black, three were Hispanic, and two were of mixed racial or ethnic background. They ranged in age from 1 to 17 years (median, 5). In 10 patients the disease was diagnosed at the onset of symptoms; in the other 29 it was diagnosed from 2 months to 12 years (median, 16 months) after the onset of symptoms.

Physicians at 28 institutions in North America and South America collaborated with the authors. Those physicians periodically provided clinical, metabolic, and developmental data, in addition to an assessment of the patients' compliance with therapy. Of the

39 patients, 7 withdrew before five years of follow-up; data on the remaining 32 are presented here.

### Therapeutic Protocols

There were three successive drug-treatment protocols,<sup>7</sup> each representing an improvement in the efficacy of waste-nitrogen excretion. Patients and physicians were offered the opportunity to adopt the modified protocols as they became available. Protocol 1 (1980 to 1984) included 11 patients (35 patient-years) who received sodium benzoate (0.25 g per kilogram of body weight per day). Protocol 2 (1984 to 1987) included 22 patients (81 patient-years) who received both sodium benzoate (0.25 g per kilogram per day) and sodium phenylacetate or sodium phenylbutyrate (0.25 to 0.30 g per kilogram per day). Protocol 3 (1987 to 1996) included 28 patients (165 patient-years) who received sodium phenylbutyrate (0.45 to 0.60 g per kilogram per day or 9.9 to 13.0 g per square meter of body-surface area per day). Each protocol included treatment of hyperammonemic encephalopathy with intravenous sodium benzoate and sodium phenylacetate as needed<sup>8</sup> and a reduction of dietary protein intake to the minimum recommended for the patient's age. The diets were supplemented with arginine (free base) or citrulline (174 mg per kilogram per day or 3.8 g per square meter per day); essential amino acid supplementation was not recommended.

To monitor growth and assess nutritional status, the software program Anthro, version 1.01,<sup>9</sup> was used to calculate age-specific z scores (the number of standard deviations above or below the mean) for each measurement. Descriptive statistics were calculated with SPSS for Windows, version 6.1.

### Cognitive Measurements

Standardized intelligence tests were administered to 23 girls at least twice after treatment was begun. Of the nine girls who did not undergo sequential testing, four were profoundly retarded at the time of enrollment. The tests were the Cattell Infant Intelligence Test, McCarthy Scales of Children's Abilities, Slosson Intelligence Test, Stanford-Binet Intelligence Scale (forms LM and IV), Wechsler Preschool and Primary Scale of Intelligence (WPPSI), Wechsler Intelligence Scale for Children — Revised (WISC-R), Wechsler Intelligence Scale for Children — III (WISC-III), and Wechsler Adult Intelligence Scale — Revised (WAIS-R). The results of the first test administered after the patient began treatment were used as the base-line measure of intelligence, and the most recent test results were used to assess changes in IQ. The length of time between the tests ranged from 1.1 to 11.2 years.

## RESULTS

### Survival

There were 3 deaths among the 32 patients. One patient, who was profoundly retarded and had severe cerebral palsy, died of aspiration pneumonia in the absence of hyperammonemia after 11 months of therapy. One patient died during a hyperammonemic episode at 16 years of age, four years after diagnosis; her intellectual level was average when tested after two years of treatment. Anecdotal information suggests that the ingestion of an abortifacient may have contributed to her terminal hyperammonemic episode. In the third patient the disease was diagnosed at 14 months of age; despite excellent compliance with treatment, she had numerous hyperammonemic episodes and died at 8 years of age during hospitalization for hyperammonemia unresponsive to treatment.

**Anthropometric Measurements**

The mean ( $\pm$ SD) birth weight of the patients was  $3.8 \pm 0.8$  kg. At the time of enrollment, the mean weight-for-age and height-for-age z scores were slightly below the mean derived from National Center for Health Statistics and Centers for Disease Control and Prevention reference data<sup>10</sup>; these findings were consistent with the history of poor weight gain, weight loss, and delayed growth noted at the time of diagnosis.

We calculated mean z scores for five-year age intervals (Table 1) and five-year treatment intervals (data not shown), because the age at entry varied among the patients. Both height-for-age and weight-for-age z scores for the patients in this study were below the mean (i.e., below zero) but remained relatively constant with increasing age. The z scores for weight for height were above the mean, indicating that although these patients tended to be small, their weight was appropriate for their height.

**Hyperammonemic Episodes**

After enrollment, 23 patients had episodes of hyperammonemia requiring hospitalization for intravenous sodium benzoate and sodium phenylacetate therapy (Table 2).<sup>8</sup> The patients receiving phenylacetate or phenylbutyrate (protocol 2 or 3) had fewer episodes than those receiving sodium benzoate alone (protocol 1). This observation is consistent with evidence from patients with other urea-cycle defects<sup>11</sup> and confirms that phenylacetylglutamine is superior to hippurate as a waste-nitrogen vehicle.<sup>6</sup>

Treated girls under five years of age had an average of one episode of hyperammonemia per year (Table 3). This frequency declined with increasing age, suggesting that the risk decreases during puberty. During the 29 patient-years of treatment for patients 20 years of age or older, only three hyperammonemic episodes occurred. This trend was similar for all three protocols, suggesting that the risk of hyperammonemia decreases with increasing age, independently of therapy.

There was wide variation among patients in the frequency of episodes. Nine patients had no hyperammonemic episodes after enrollment; their mean length of treatment was seven years. Twenty patients had 1 episode or less per year (mean, 0.4 episode; range, 0.1 to 1.0); their mean length of treatment was eight years. The remaining three surviving patients each had 10 or more hyperammonemic episodes; their average length of treatment was 11 years, and the frequency of episodes ranged from 1 to 3 per year.

**Cognitive Development**

At the time of initial testing, the IQ scores of the 23 girls in whom intelligence was tested longitudi-

**TABLE 1. ANTHROPOMETRIC MEASUREMENTS IN GIRLS WITH ORNITHINE TRANSCARBAMYLASE DEFICIENCY, ACCORDING TO AGE.\***

MEASUREMENT	NO. OF PATIENTS	MEAN TIME AFTER INITIATION OF TREATMENT (YR)	NO. OF MEASUREMENTS	MEAN z SCORE
<b>Height-for-age z-score distribution</b>				
<5 yr	16	1.4 $\pm$ 0.9	39	-0.7 $\pm$ 1.2
5-9.9 yr	20	3.8 $\pm$ 1.9	66	-0.9 $\pm$ 0.9
10-14.9 yr	15	7.6 $\pm$ 3.3	62	-0.6 $\pm$ 1.0
15-19.9 yr	13	7.6 $\pm$ 4.1	28	-0.7 $\pm$ 0.8
<b>Weight-for-age z-score distribution</b>				
<5 yr	17	1.4 $\pm$ 0.9	43	-0.6 $\pm$ 1.6
5-9.9 yr	21	3.9 $\pm$ 2.0	73	-0.4 $\pm$ 1.2
10-14.9 yr	15	7.5 $\pm$ 3.3	65	-0.2 $\pm$ 1.1
15-19.9 yr	13	7.2 $\pm$ 4.1	28	-0.1 $\pm$ 1.2
<b>Weight-for-height z-score distribution†</b>				
<5 yr	15	1.4 $\pm$ 0.9	38	0.1 $\pm$ 1.4
5-9.9 yr	20	3.8 $\pm$ 1.9	60	0.5 $\pm$ 1.3

\*Plus-minus values are means  $\pm$ SD.

†Weight-for-height z scores are calculated from birth to the age of 10 years.<sup>8</sup>

**TABLE 2. HYPERAMMONEMIC EPISODES DURING TREATMENT OF GIRLS WITH ORNITHINE TRANSCARBAMYLASE DEFICIENCY, ACCORDING TO THERAPEUTIC PROTOCOL.\***

THERAPEUTIC PROTOCOL	NO. OF PATIENTS	NO. OF HYPERAMMONEMIC EPISODES	PATIENT-YEARS OF TREATMENT	FREQUENCY (EPISODES/PATIENT-YEAR)
1	11	25	35	0.7
2	22	32	81	0.4
3	28	76	165	0.5
Total	61	133	281	0.5

\*Data for Patient 3 were omitted from the calculations because she had more than 20 hyperammonemic episodes during treatment in protocols 1 and 2, despite excellent compliance.

**TABLE 3. HYPERAMMONEMIC EPISODES DURING TREATMENT OF GIRLS WITH ORNITHINE TRANSCARBAMYLASE DEFICIENCY, ACCORDING TO AGE.\***

AGE (YR)	NO. OF PATIENTS	NO. OF HYPERAMMONEMIC EPISODES	PATIENT-YEARS OF TREATMENT	FREQUENCY (EPISODES/PATIENT-YEAR)
<5	16	39	40	1.0
5-9.9	21	44	81	0.5
10-14.9	19	29	75	0.4
15-19.9	18	18	57	0.3
$\geq$ 20	8	3	29	0.1
Total	82	133	281	0.5

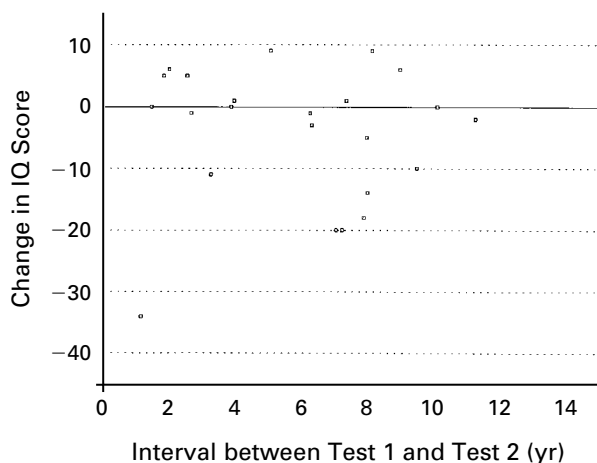
\*Data for Patient 3 were omitted from the calculations because she had more than 20 hyperammonemic episodes during treatment in protocols 1 and 2, despite excellent compliance.

nally ranged from 56 to 111 (mean, 84), with 10 girls having scores below 80. At follow-up, their scores ranged from 36 to 116. The correlation between the IQ scores at initial testing and follow-up was 0.9 ( $P < 0.001$ ). Of the 23 girls, only 4 had changes in IQ that exceeded 15 points (the equivalent of 1 SD); these were all decreases. The change in IQ was not affected by the length of the interval between tests, the age at first testing, the initial IQ, or the number of years of treatment. The change in IQ in relation to the interval between tests is shown in Figure 2.

Scores on the verbal and performance subtests were compared in the 13 girls who had these tests more than once. At initial testing, 6 of the 13 had significantly higher verbal than performance scores. The differences ranged from 14 to 23 points; a difference of 15 points was statistically significant ( $P = 0.01$ ). All six girls had a narrowing of the verbal–performance difference on their second test, as did two other girls whose verbal–performance difference was small at initial testing. Greater verbal–performance differences were not related to the age at first testing or to the overall IQ score.

### DISCUSSION

We report here on the long-term outcome of symptomatic girls with ornithine transcarbamylase deficiency who were treated with drugs that provide an alternative vehicle for waste-nitrogen excretion. Each had had at least one episode of potentially life-threatening encephalopathy before enrollment, and



**Figure 2.** Change in IQ Score for 23 Girls with Ornithine Transcarbamylase Deficiency Who Had at Least Two Intelligence Tests after Enrollment in the Treatment Protocols.

Each point represents the change in IQ for one girl during the time indicated on the abscissa. At initial testing the girls ranged in age from 2 to 18 years, and their IQ scores ranged from 56 to 111. At follow-up testing, their scores ranged from 36 to 116.

all presumably remained vulnerable to episodic hyperammonemia. The goal of treatment was to improve survival and maintain cognitive functioning by reducing the risk of hyperammonemic episodes.

The authors, funding agencies, and the Johns Hopkins Joint Committee on Clinical Investigations all agreed that ethical issues precluded a controlled clinical trial to compare the outcomes in treated and untreated girls. Thus, there are no studies of untreated girls with symptomatic ornithine transcarbamylase deficiency with which these results can be compared. However, historical data suggest a high rate of mortality from coma among untreated carrier females<sup>12</sup>; among a selected group from 17 pedigrees, 9 deaths occurred during 11 episodes of coma (82 percent mortality). In our study over 90 percent of the girls treated according to these protocols survived for at least five years, suggesting that the treatment reduced the frequency of hyperammonemic encephalopathy and the consequent risk of death.

Treatment with sodium phenylacetate or sodium phenylbutyrate, which activates the synthesis of phenylacetylglutamine containing two waste-nitrogen atoms (Fig. 1), decreased the frequency of hyperammonemic episodes by approximately 40 percent, as compared with treatment with sodium benzoate, which activates the synthesis of a product containing only one waste-nitrogen atom. This suggests that treatment that improves waste-nitrogen excretion stabilizes plasma ammonium concentrations and reduces the frequency of hyperammonemic episodes in these patients. Although it would have been desirable to compare the frequency of episodes before and after treatment began, such a comparison was hampered both by the wide phenotypic variation among these patients and by the difficulties of retrospectively interpreting physicians' and parents' descriptions of lethargy, irritability, and sleepiness.

Historical data suggest that subtle cerebral damage may occur in untreated carriers, despite the absence of overt symptoms.<sup>4</sup> Our findings that four patients were profoundly retarded at or shortly after diagnosis and that the initial mean IQ score among those tested repeatedly was only 84 (low average) support the hypothesis that many, if not all, untreated symptomatic girls are on a trajectory of cognitive decline. Our analysis of intelligence-test performance clearly indicates that intellectual functioning has remained stable for more than 80 percent of the 23 girls enrolled in these treatment protocols in whom intelligence was tested longitudinally. Further, the change in IQ in three of the four girls who had a substantial decline may be a testing artifact, resulting in part from use of two different forms of the Stanford–Binet test (LM and IV)<sup>13</sup> and Wechsler Intelligence Scale for Children (WISC-R and WISC-III).<sup>14</sup>

Although these results suggest that treatment that

reduces the risk of hyperammonemic episodes is effective in improving survival and stabilizing cognitive functioning, the treatment outcome for any individual girl cannot be predicted and is most likely due to a combination of unmeasurable factors, including residual ornithine transcarbamylase activity, the degree of adherence to a low-protein diet, the degree of compliance with the therapeutic regimen, environmental stresses, and genetic factors. It is also important to note that our results are limited to girls whose symptoms brought them to the attention of a referral center; whether ostensibly normal heterozygous girls might benefit from treatment is not known.

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Dr. Brusilow has an indirect equity interest in and is an employee of Uceclid Pharma, which will be marketing sodium phenylbutyrate. He also receives royalties from the sales of sodium benzoate-sodium phenylacetate (Ucephan, McGaw).

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## REFERENCES

1. Msall M, Batshaw ML, Suss R, Brusilow SW, Mellits ED. Neurologic outcome in children with inborn errors of urea synthesis: outcome of urea cycle enzymopathies. *N Engl J Med* 1984;310:1500-5.
2. Brusilow SW. Urea cycle disorders: clinical paradigm of hyperammonemic encephalopathy. In: Boyer JL, Ockner RK, eds. *Progress in liver disease*. Vol. 13. Philadelphia: W.B. Saunders, 1995:293-309.
3. Finkelstein JE, Hauser ER, Leonard CO, Brusilow SW. Late-onset ornithine transcarbamylase deficiency in male patients. *J Pediatr* 1990;117:897-902. [Erratum, *J Pediatr* 1991;118:326.]
4. Batshaw ML, Roan Y, Jung AL, Rosenberg LA, Brusilow SW. Cerebral dysfunction in asymptomatic carriers of ornithine transcarbamylase deficiency. *N Engl J Med* 1980;302:482-5.
5. Rowe PC, Newman SL, Brusilow SW. Natural history of symptomatic partial ornithine transcarbamylase deficiency. *N Engl J Med* 1986;314:541-7.
6. Brusilow SW, Finkelstein JE. Restoration of nitrogen homeostasis in a man with ornithine transcarbamylase deficiency. *Metabolism* 1993;42:1336-9.
7. Brusilow SW, Maestri NE. Urea cycle disorders: diagnosis, pathophysiology, and therapy. *Adv Pediatr* 1996;43:127-70.
8. Brusilow SW, Danney M, Waber LJ, et al. Treatment of episodic hyperammonemia in children with inborn errors of urea synthesis. *N Engl J Med* 1984;310:1630-4.
9. Sullivan KM, Gorstein J. ANTHRO: software for calculation of pediatric anthropometry, version 1.01. Atlanta: Centers for Disease Control, 1990 (software and manual).
10. Dibley MJ, Goldsby JB, Staehling NW, Trowbridge FL. Development of normalized curves for the international growth reference: historical and technical considerations. *Am J Clin Nutr* 1987;46:736-48.
11. Maestri NE, Clissold DB, Brusilow SW. Long-term survival of patients with argininosuccinate synthetase deficiency. *J Pediatr* 1995;127:929-35.
12. Batshaw ML, Msall M, Beaudet AL, Trojak J. Risk of serious illness in heterozygotes for ornithine transcarbamylase deficiency. *J Pediatr* 1986;108:236-41.
13. Sattler JM. *Assessment of children revised and updated*. 3rd ed. San Diego: JM Sattler, 1992.
14. Wechsler D. *Manual for the Wechsler Intelligence Scale for Children*. 3rd ed. San Antonio, Tex.: Psychological Corporation, 1991.