Neurodevelopmental Outcome and Treatment Efficacy of Benzoate and Dextromethorphan in Siblings with Attenuated Nonketotic Hyperglycinemia

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Objective To evaluate the impact of sodium benzoate and dextromethorphan treatment on patients with the attenuated form of nonketotic hyperglycinemia.

Study design Families were recruited with 2 siblings both affected with attenuated nonketotic hyperglycinemia. Genetic mutations were expressed to identify residual activity. The outcome on developmental progress and seizures was compared between the first child diagnosed and treated late with the second child diagnosed at birth and treated aggressively from the newborn period using dextromethorphan and benzoate at dosing sufficient to normalize plasma glycine levels. Both siblings were evaluated with similar standardized neurodevelopmental measures.

Results In each sibling set, the second sibling treated from the neonatal period achieved earlier and more developmental milestones, and had a higher developmental quotient. In 3 of the 4 sibling pairs, the younger sibling had no seizures whereas the first child had a seizure disorder. The adaptive behavior subdomains of socialization and daily living skills improved more than motor skills and communication.

Conclusions Early treatment with dextromethorphan and sodium benzoate sufficient to normalize plasma glycine levels is effective at improving outcome if used in children with attenuated disease with mutations providing residual activity and when started from the neonatal period. *(J Pediatr 2016;170:234-9)*.

Nonketotic hyperglycinemia (NKH) is a neurometabolic disorder caused by deficient activity of the glycine cleavage enzyme system. Classic NKH is caused by mutations in either the *GLDC* gene, encoding for the P-protein, or the *AMT* gene, encoding for the T-protein.[1,2] Patients with severe NKH present neonatally or in early infancy with lethargy progressing to coma, transient apnea, and myoclonic movements. Patients develop intractable seizures, axial hypotonia, and limb spasticity, and make no developmental progress.[3] In contrast, patients with attenuated NKH make variable developmental progress resulting in mild to severe intellectual disability, no or treatable epilepsy, and mild to no spasticity.[3,4] They often have chorea, as well as episodes of lethargy and ataxia. The primary determinant of outcome in attenuated NKH is the genetic mutation, which determines the amount of residual enzyme activity remaining.[5] Other factors also contribute to outcome, and a role for treatment has been hypothesized.[1,5,6]

Current treatment consists of reducing the glycine levels with benzoate and blocking the effect of excess glycine on the N-methyl-D-aspartate (NMDA) receptors with dextromethorphan. Benzoic acid is activated to benzoyl-CoA, then is conjugated with glycine to form hippurate, which is excreted in the urine, thus, eliminating glycine and reducing glycine levels.[7] The benzoate dose is individually tailored with the aim of plasma glycine levels between 120 and 300 μM. The required benzoate dose depends on the residual glycine cleavage enzyme activity. It is lower in attenuated NKH than in severe NKH, as reflected in a lower glycine index.[8] Glycine is an allosteric co-activator of the NMDA-type

AE	Age equivalent
BASC	Behavior Assessment System for Children
DQ	Developmental quotient
Mullen scales	Mullen Scales of Early Learning
NKH	Nonketotic hyperglycinemia
NMDA	N-methyl-D-aspartate
WPPSI	Wechsler Preschool and Primary Scale of Intelligence

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glutamate receptor, whose overstimulation has been posed as a component of the pathogenesis of NKH.[9,10] Dextromethorphan is a weak inhibitor of this receptor, and its use can decrease seizures in NKH.[10-12]

Treatment with sodium benzoate has been shown to improve alertness allowing for more learning opportunities, and to decrease the propensity for seizures.[3,7,8,13,14] Treatment with dextromethorphan improved seizures, and in some patients appeared to improve alertness and outcome.[10,11,15,16] Yet, for many patients, combined treatment with both benzoate and dextromethorphan failed to improve outcome.[15,17-19] In patients with severe NKH, even when started in the early neonatal period, treatment does not improve developmental outcome, which remains severely impaired, and does not prevent the development of severe therapy-resistant epilepsy, although treatment may improve overall seizure control.[17,18] These results have resulted in justifiable skepticism about the benefits of this treatment. However, previous studies have failed to take into account the substantial genetic heterogeneity of NKH. Genetic mutations have a strong impact on outcome in NKH,[5] and large intragenic genetic heterogeneity complicates evaluating the impact of a treatment intervention in NKH. Patients with 2 mutations without any residual activity always have severe NKH, make no developmental progress, and have a severe seizure disorder, regardless of treatment even when initiated from birth.[17] However, patients with at least 1 missense mutation have variable degrees of developmental progress. However, in attenuated NKH, case reports have suggested that treatment may be beneficial, but the response may be modified by the genetic mutation and by the timing of initiation of therapy with treatment in the first 2 years of life being critical.[4,6,19,20] Patients with mild mutations such as p.Ala802Val made variable to excellent neurodevelopmental progress with treatment but not when untreated.[6]

The large genetic heterogeneity makes controlled studies of outcome difficult. To control for genetic heterogeneity, we studied 4 sibling pairs with attenuated NKH and with mutations that confer residual activity. In these 4 sibling pairs, we compared the effect on neurodevelopmental outcome of early vs delayed treatment.

Methods

The study was approved by the Colorado Multiple Institutional Review Board (COMIRB 05-0790), and written informed consent was obtained from subjects' parents. Families were identified where there were 2 siblings both affected with attenuated NKH, and where the first sibling was treated late and the subsequent sibling was treated early. Data on clinical history, treatment history, and brain magnetic resonance imaging were reviewed. Specific attention was paid to the timing and effectiveness of treatment, and presence of seizures and antiepileptic medications used. Benzoate treatment was considered effective when blood glycine levels were controlled in the normal range,[8] and dextromethorphan was used at a dose of 3-12 mg/kg/d. All patients were examined by a metabolic physician (J.H. or J.C.) and by a pediatric neuropsychologist (K.B. or A.H.). The genetic mutations were identified in each sibling pair by sequencing of the *GLDC* gene.[5] The residual activity of the mutation was determined by expression of the mutation in COS cells and measurement of the activity using the glycine exchange reaction in the presence of recombinant lipoylated human H-protein, and reported as percentage of the wild type P-protein expressed in the same experiment as previously described.[5] The glycine index was calculated by subtracting the molar glycine intake in food from a dietary recall from the molar dose of sodium benzoate needed to normalize plasma glycine levels divided by body weight, which reflects a whole body balance of glycine metabolism.[8]

Three sets of siblings were evaluated at Children's Hospital Colorado and University of Colorado including age-appropriate neuropsychological evaluations; the fourth family was evaluated in Sydney, Australia. The primary outcome was overall neurocognitive ability. Different scales were used in each family because of different ages and developmental levels. At the University of Colorado, children under the age of 6 years were given the Mullen Scales of Early Learning (Mullen scales).[21] Children older than 6 years of age were administered one of the Wechsler tests (Wechsler Intelligence Scale for Children, Fourth Edition[22] or Wechsler Preschool and Primary Scale of Intelligence [WPPSI][23]). All measures indicated full-scale IQ scores with a mean of 100 and a SD of 15 representing the average range of functioning and scores more than 2 SDs below the mean (ie, <70) representing impairment. Because of the extent of their impairments, the WPPSI, Third Edition was used in families 1 and 3,[23] and the Mullen scales used in family 2.[21] The Mullen scales can be administered to infants and children up to 68 months of age as a measure of development. An Early Learning Composite score (full scale IQ), and age-equivalents can be computed separately for the 5 scales: gross motor, visual reception, fine motor, expressive language, and receptive language.[16] The WPPSI, Third Edition is a measure of verbal and nonverbal reasoning abilities for preschool children.[23] Patient 3B also completed an educational evaluation, using the Battelle Developmental Inventory, Second Edition, which is a standardized battery that assesses children in 5 domains: motor, cognitive, personal-social, adaptive, and communication.[24] In Sydney, at the age of 4 years family 4 was administered the Differential Ability Scales, Second Edition, which is a nationally normed, individually administered battery of cognitive and achievement tests for a range of developmental levels.[25] At age 8 years 3 months, family 4 completed the Wechsler Intelligence Scale for Children, Fourth Edition.[17] For each child, a General Developmental Quotient was calculated. The developmental quotient (DQ) is a measure of the rate of development. By using the mean or median age at which a

milestone presents, a functional age equivalent (AE) for a child's development is given. DQ is the ratio of developmental AE to chronological age.

As a second outcome measure, in some families additional testing was done to evaluate adaptive behavior, and visuomotor, language, and emotional and behavioral functioning. The Vineland Adaptive Behavior Scales were used to measure daily functioning.[26] The Vineland Adaptive Behavior Scale is a widely used norm-referenced parent report measure of personal and social sufficiency in the areas of communication, daily living skills, socialization, and motor function. These domain areas are combined to form an adaptive behavior composite score. Similar to IQ scores, domain areas and the composite score have a mean of 100 and a SD of 15 with 2 SDs below the mean representing clinical impairment. Visuomotor function was assessed by the Beery-Buktenica Test of Visual Motor Integration.[27] Receptive and expressive language was assessed by Peabody Picture Vocabulary Test, Fourth Edition,[28] or the Clinical Evaluation of Language Fundamentals–Preschool, Second Edition.[29]

Emotional and behavioral components were evaluated with the Behavior Assessment System for Children (BASC), Second Edition. The BASC, Second Edition is a standardized rating scale that compares the child's emotional and behavioral functioning to that of peers of the same age using t scores with a mean of 50 and SD of 10.[30]

Results

In 4 families, the first child was diagnosed and treated after 2-6 months, whereas the second child was diagnosed prenatally or neonatally and treated effectively from the first week with benzoate and dextromethorphan. The diagnosis, symptoms, and developmental milestones are listed in Table I.

Families 1, 2, and 3 were compound heterozygous for a mutation affecting a conserved splice site, and a missense mutation (Table II). Expression of the missense mutations showed residual activities of 18.2%, 25.8%, and 11.9% of wild type for p.Ala202Val, p.Ala802Val, and p.Ala389Val respectively. Family 4 was homozygous for a missense mutation resulting in p.Cys291Tyr, which had 1.1% of wild type residual activity (Table II). The results of neurodevelopmental evaluations are listed in Table III.

Family 1

Patient 1A was tested at age 11 years, 2 months with a DQ 37 and an AE of 4 years, 1 month. Her receptive language was at 4 years, 8 months and expressive language at 5 years, 7 months level. Visual motor integration skills were less developed at an AE score of 3 years, 5 months. For adaptive behaviors, her overall composite score was in the moderately impaired range with a personal strength in socialization and significant impairment in motor skills. She was echolalic, and very fidgety, with intermittent attention. She demonstrated affection.

Patient 1B was evaluated at age of 9 years resulting in a DQ of 51 with an AE of 4 years, 7 months. Receptive language was at 6 years, 2 months and expressive language at 4 years, 7 months level. Visual motor integration skills were in the moderately impaired range with an AE score of 4 years, 6 months. In terms of adaptive behaviors, his overall composite score was in the borderline range with AE scores between 7 years, 6 months for communication, daily living skills, and socialization, and only 1 year, 4 months for gross motor skills. In terms of behavior, he was attentive but sometimes impulsive, requiring additional time for processing instructions. He was interactive but echolalic.

Family 2

Patient 2A was evaluated at age 4 years, 9 months resulting in a DQ <20 in the markedly impaired range with an AE of 9 months. On the BASC, Preschool Version, his parents rated his overall behavior in the moderately impaired range. On the Vineland Adaptive Behavior Scales, his overall composite score indicated he was markedly impaired with AE scores ranging from less than 1 month to 8 months.

Patient 2B was evaluated at age 2 years, 5 months with a DQ of 48. AE scores were 14 months for gross motor skills, 15 months for visual reception, 17 months for fine motor skills, 11 months for receptive language, and 8 months for expressive language. For adaptive behavior, his overall composite score indicated mildly impaired adaptive functions with AE scores ranging from 7 months to 1 year, 6 months.

Family 3

Patient 3A was evaluated at the age of 5 years resulting in overall cognitive abilities in the moderately impaired range with a full scale IQ of 44, with consistent verbal and performance cognitive abilities. Overall language abilities were in the moderately impaired range with a standard score of 45. In terms of adaptive behaviors, her overall composite score was less developed and in the markedly impaired range (standard score of 35), with AE scores ranging from less than 1 month (communication) to 8 months (daily living skills and motor skills).

Patient 3B was evaluated at the age of 14 months with an overall DQ of 30. Adaptive behaviors were in the moderately impaired range with an overall standard score of 51. At the age of 2 years, 11 months, she completed an educational evaluation receiving an overall standard score of 55 in cognitive abilities, with comparative adaptive development (standard score of 55) with AEs ranging from 6 months to <24 months.

Family 4

Patient 4A appeared normal until he developed a rapidly worsening epileptic encephalopathy at age 6 months. His seizures became intractable; he lost all developmental skills, and died at age 13 months. His developmental status was never formally tested. Patient 4B had a cognitive assessment at age 4 years, 4 months with an overall IQ of 78. He had mild to moderate receptive language and severe expressive language deficit. At age 6 years, on the Clinical Evaluation

Table I. Clinical findings in 4 siblings pairs with attenuated NKH

	Case 1A	Case 1B	Case 2A	Case 2B	Case 3A	Case 3B	Case 4A	Case 4B
Clinical presentation and diagnosis								
Presentation	1 d	1 d	1 d		5 d	3 mo	6 mo	
Diagnosis	2 mo	Prenatal	6 mo	Prenatal	1 mo	Prenatal	6 mo	Neonatal
Symptoms	Hypotonia, lethargic, myoclonic jerks	Hypotonia	Seizures	Delayed development		Lethargy, seizures	Seizures, regression	Delayed development
Biochemical findings at diagnosis and treatment								
Glycine, plasma (μM)*	578 (<436)	294 (<540)	552 (<436)	693 (<540)	637 (<436)	536 (<540)	961 (<436)	713 (<540)
Glycine, CSF (μM)*	89 (<20)	NA	103 (<12)	85 (<20)	136 (<20)	NA	52 (<12)	47 (<20)
CSF:Plasma glycine*	0.15 (<0.02)	NA	0.17 (<0.02)	0.12 (<0.02)	NA	NA	0.05 (<0.02)	0.07 (<0.02)
Treatment achieved	6 mo	First wk	7 mo	First wk	6-8 wk	First wk	6 mo	First wk
Glycine index (mmol/kg/d)	2.77	2.65	2.30	1.84	2.52	0.68	NA	NA
Developmental milestones								
Reaching/grasping	9 mo	6 mo	2-3 y	9 mo	6 y	1 y	5 mo	3 mo
Independent sit	1 y 8 mo	7 mo	3 y	1 y 5 mo	2.5 y	1 y 3 mo	No	10 mo
Independent walk	5 y	2 y 6 mo	Stand only (4 y)	1 y 10 mo	With walker only	With walker only		14 mo
First words	1 y 11 mo	1 y 4 mo	6 y (1 word)	3 y 6 mo	No words	14 mo (few)		16 mo
Clinical evaluation								
Age at evaluation	11 y 2 mo	9 y	4 y 10 mo	2 y 5 mo	6 y 6 mo (deceased, 7 y)	14 mo	NA (deceased, 13 mo)	5 y 7 mo
ADHD	Yes	Yes	No	No	No	No		Yes
Chorea	Yes (mild)	Yes (mild)	Yes	Yes	Yes	No		No
Dysmetria	Yes (mild)	Yes (mild)	No	No	No	No		No
Hyperreflexia	Yes	Yes	Yes	No	Yes	No		No
Temper tantrums	Yes	No	No	Yes	No	No		No

NA, not available; *CSF*, cerebrospinal fluid; *ADHD*, attention deficit hyperactivity disorder.
The biochemical diagnostic findings, symptoms, and developmental milestones are provided.
*Normal values are in parenthesis.

of Language Fundamentals Fourth Edition, he had a core language index standard score of 63 with only word structure at the lowest end of the normal range, and great difficulties with concepts and following directions, recalling sentences, and formulating sentences. A repeat cognitive assessment at age 8 years, 3 months was consistent with his earlier assessment. Overall, he functioned in the borderline range of intellectual ability (IQ of 75). He had difficulty with all aspects of attention and was easily distracted. Areas of personal strength were in motor speed, word reading, and spelling, which were average.

Discussion

The overall goal of neurodevelopmental evaluations is to identify brain and behavior abnormalities that can be sensitive to changes resulting from treatment. Neurodevelopmental testing serves as a sensitive measure of disease severity and can be used to measure disease progression and treatment outcome. Quantified comparisons with age mates allows for measurement of the severity of deficits. Here we report detailed neurodevelopmental testing in siblings with attenuated NKH and use this measure to indicate the impact of treatment. Because of a wide range of developmental levels, different instruments were used; however, within the same family similar rating instruments were used for both siblings thus allowing a direct comparison between them.

In this study, we aimed to evaluate whether current treatment with sodium benzoate and dextromethorphan influenced neurocognitive outcome specifically in those patients with attenuated NKH. To control for genetic heterogeneity, we comparatively studied pairs of patients with similar mutations such as siblings, similar to what

Table II. Mutations and residual enzyme activities

Family	Family 1	Family 2	Family 3	Family 4
Paternal mutation	c. 2665+1G>C	c.2665+1G>A	c.1166C>T	c.872G>A
Effect	p.IVS22+1G>C, missplicing	p.IVS22+1G>A, missplicing	p.Ala389Val, missense	p.Cys291Tyr, missense
Maternal mutation	c. 605C>T	c.2405C>T	c.1580+2T>G	c.872G>A
Effect	p.Ala202Val, missense	p.Ala802Val, missense	p.IVS12+2T>G, missplicing	p.Cys291Tyr, missense
Residual activity of missense mutation*	18.2% $\pm$ 3.43%	25.8% $\pm$ 4.4%	11.9% $\pm$ 2.53%	1.1% $\pm$ 0.33%

The mutations present in each family and the residual activity of the expressed missense mutation are provided.
*Residual activity values are expressed relative to the mean of normal in assay control and SD of replicate assays.

Table III. Comparison of sibling pairs on developmental outcome

	1A	1B	2A	2B	3A	3B	4A	4B
Sex	Female	Male	Male	Male	Female	Female	Male	Male
Age at evaluation	11 y 2 m	9 y	4 y 9 m	2y 5 m	5 y	1 y 2 m	Deceased	8 y 3 m
Seizures	6 w to 4 y	Never	6 w to 6 y	Never	From 4 d	From 3 m	From 6 m	Never
IQ or GDQ	DQ 37	DQ 51	DQ <20	DQ 48	IQ 44	IQ 55	-	IQ 75
Adaptive functions								
Communication	54	77	42	69	40	52	-	Not given
Daily living	59	71	40	67	38	21		
Socialization	66	73	46	74	48	82		
Motor skills	*	*	37	72	28	57		
Composite	59	72	40	67	35	51		
BASC BSI t score	63	62	54	53				

BSI, Behavioral Symptoms Index; *GDQ*, General Developmental Quotient.
The results of cognitive testing, using testing as outlined in the text for each family, are provided as either an IQ or as a GDQ. The standard scores on the Vineland Adaptive Behavior Scales with subdomain scores. The BASC, Second Edition BSI t scores are reported with average range of 40-60.
*Not able to evaluate based on performance of the national standardization sample of the items of the motor skills domain, which are appropriate for individuals through ages <6 y.

had been used to evaluate the impact of treatment intervention in an equally genetically heterogeneous condition such as pyruvate dehydrogenase deficiency.[31] Each family had 1 allele carrying a mutation that resulted in residual activity, thus, allowing at least the potential for developmental progress. In families 1-3, the second allele was a consensus splice site mutation with likely no effective protein made, and the potential confounding effect of a dominant negative missense mutation was avoided. We showed in these 4 kindreds that the child who was treated from birth with a combination of benzoate sufficient to control glycine levels (<400 μM) and with dextromethorphan (≥3 mg/kg/d for at least the first 2 years) progressed substantially better than their siblings who had been late treated. This was evident on cognitive abilities, communication skills, and adaptive skills, but not on the emotional and behavioral scale, either through standardized testing or during clinical interviewing. Almost all patients had chorea and hyperactivity regardless of treatment. In each family, the second sibling, who had been treated since the neonatal period, achieved milestones earlier and acquired more skills than the first sibling. Seizures were less frequent and less severe in the second sibling in 3 of 4 families. Similar to what was noted previously, there did not appear to be a relation between the amount of residual activity in the expression results and outcome.[5] Patient 4B with the best outcome had the lowest residual activity, but was the only patient with 2 alleles with residual activity.

Thus, some residual activity is required for developmental progress to be possible. In this setting, early treatment in the first weeks to months of life is important to obtain optimal results of current therapeutic intervention. Hypotheses for this critical time vulnerability include the ontogeny of neurotransmitter receptors such as the NMDA receptor[6,12,32] or the prevention of irreversible early brain damage.[20] Regardless of the underlying mechanism, these data indicate that for patients with attenuated NKH, when the patient carries at least 1 mutation that confers residual activity, it is important for optimal outcome to initiate therapy promptly and to maintain this throughout the early years. This study does not make it possible to distinguish whether 1 component of this therapy was more important than the other, or for how long therapy should be continued. In the study by Korman et al,[6] therapy was discontinued without adverse effect in one patient after 2 years, but other patients have experienced adverse effects upon discontinuation of treatment.[14] Both dextromethorphan and ketamine have been used as a partial NMDA receptor antagonist in NKH, and which treatment or any other NMDA receptor antagonist is more effective remains to be evaluated.[6,12,20,33]

We conclude that in attenuated NKH, treatment with sodium benzoate and dextromethorphan improves neurocognitive outcome when instituted early. This is in contrast to the lack of efficacy in those patients with severe NKH. For patients in whom mutations conferring residual activity are identified, therapy should be pursued early and strictly to allow optimal outcome. Treatment may be a modifying factor of the genotype-phenotype relation in patients with attenuated NKH. ■

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