J Inherit Metab Dis (2012) 35:253–261
DOI 10.1007/s10545-011-9398-1

ORIGINAL ARTICLE

Prediction of long-term outcome in glycine encephalopathy: a clinical survey

Julia B. Hennermann · Jeanne-Marie Berger · Ulrike Grieben · Gunter Scharer · Johan L. K. Van Hove

Received: 12 May 2011 / Revised: 18 August 2011 / Accepted: 13 September 2011 / Published online: 15 October 2011

Abstract
Objective Glycine encephalopathy (GE) is a rare autosomal recessive inborn error of glycine degradation resulting in severe encephalopathy with ensuing poor outcome. Attenuated variants with a significantly better outcome have been reported. Early prediction of long-term outcome is not yet possible.
Methods We compared the clinical and biochemical features of 45 children, each with a different course of the disease, to help determine predictors of long-term outcome.
Results The most common presenting symptoms were hypotonia, seizures, and coma. In this study, 85% of the patients presented within the first week of life, and 15% presented after the neonatal period up to the age of 12 months. Developmental progress was made by 19% of those children presenting during the neonatal period and by 50% of those presenting in infancy. Initial CSF and plasma glycine concentrations were not useful in differentiating severe and attenuated outcome. A severe outcome was significantly associated with early onset of spasticity, frequent hiccupping, EEG burst-suppression or hypsarrhythmia patterns, microcephaly, and congenital or cerebral malformations, e.g. corpus callosum hypoplasia. An attenuated outcome was significantly associated with hyperactivity and choreiform movement disorders. We describe a severity score which facilitates the prediction of the outcome in patients with GE.
Conclusion Prediction of the outcome of GE may be facilitated by recognizing selected clinical parameters and early neuroimaging findings.

Communicated by: Sylvia Stoeckler-Ipsiroglu

Competing interests: None declared.

Electronic supplementary material The online version of this article (doi:10.1007/s10545-011-9398-1) contains supplementary material, which is available to authorized users.

J. B. Hennermann (✉) · J.-M. Berger
Department of Pediatrics, Charité Universitätsmedizin,
Augustenburger Platz 1,
13353 Berlin, Germany
e-mail: julia.hennermann@charite.de

J.-M. Berger
Department of Child and Adolescent Psychiatry,
Psychosomatics and Psychotherapy,
Medizinische Einrichtungen des Bezirks Oberpfalz,
Regensburg, Germany

U. Grieben
Department of Neuropediatrics, Charité Universitätsmedizin,
Berlin, Germany

U. Grieben
Experimental and Clinical Research Center,
A Joint Cooperation Between Charité Medical Faculty
and the Max-Delbrück Center for Molecular Medicine,
Berlin, Germany

G. Scharer · J. L. K. Van Hove
Department of Pediatrics, University of Colorado,
Aurora, CO, USA

J. L. K. Van Hove
Department of Pediatrics, Katholieke Universiteit Leuven,
Leuven, Belgium

Abbreviations

GCS	Glycine cleavage system
GE	Glycine encephalopathy
NMDA	N-methyl-D-aspartate

Introduction

Glycine encephalopathy (GE, synonym: nonketotic hyperglycinemia, OMIM 238,300) is an autosomal recessive inborn error of glycine degradation resulting in an excessive accumulation of glycine in all tissues, particularly in the central nervous system. The underlying defect is a deficiency of the glycine cleavage system (GCS), an intramitochondrial enzyme complex made up of four different protein components: the P-, T-, H- and L-proteins (Hamosh and Johnston 2001). Up to 80% of children have a defect in the P-protein, 20% in the T-protein, and only a few, a deficient H-protein. The L-protein seems not to be affected in GE (Applegarth and Toone 2001, 2004; Tada and Kure 1993).

Glycine acts both as an excitatory and an inhibitory neurotransmitter. It has an excitatory effect at the N-methyl-D-aspartate (NMDA) receptor channel complex located in the cortex. Overstimulation at these receptors may cause intractable seizures and brain damage (Tada and Kure 1993). Stimulation of the glycinergic receptors located in the spinal cord and the brain stem has an inhibitory effect, resulting in neonatal apnea, hypotonia, and hiccupping (Hamosh and Johnston 2001). Stimulation of these same glycinergic receptors on neural progenitor cells has an excitatory effect and could be involved in the malformations sometimes observed in patients with GE (Nguyen et al. 2001). Furthermore, glycine has been shown to induce bioenergetical dysfunction by inhibiting parts of the citric acid cycle and the respiratory chain, and by reducing the activity of Na^+,K^+ATPase and mitochondrial creatine kinase activities (Busanello et al. 2010).

The prevalence of GE is estimated at 1:60.000 (Hamosh and Johnston 2001; Applegarth et al. 2000). Most patients present within the first days of life with lethargy, hypotonia, seizures, and apnea, leading to early death or various degrees of mental retardation and intractable seizures. A subset of patients, however, present in the neonatal period or early infancy and make developmental progress (Hoover-Fong et al. 2004; Dinopoulos et al. 2005). Early prediction of the outcome of the disease is not yet possible. An effective treatment does not exist. Current therapy is aimed at decreasing glycine concentrations and blocking the effect of glycine at neurotransmitter receptors. Glycine plasma concentrations can be reduced by benzoate and low protein diet. NMDA receptor antagonists include dextromethorphan, ketamine, and felbamate. Combined anticonvulsive treatment is necessary, but often ineffective in children with severe GE. To date no recommendations for anticonvulsive therapy in GE exist.

This study detailed clinical findings in a series of patients with GE. This allowed the identification of parameters that predict the outcome in patients surviving the neonatal period. We compared the clinical, laboratory, and therapeutic aspects of the different forms of GE as they relate to long-term outcome.

Methods and patients

Patient cohort

Comprehensive data on 45 children from 42 families with GE, all Caucasian, were collected in hospitals in Germany, Belgium, The Netherlands, and Luxemburg. There was an almost even number of boys (n=24) and girls (n=21). Most families were of German (62%) or Turkish origin (24%); other families were of Belgian, Dutch, Italian, and Swedish origin. Ten families were consanguineous. The diagnosis of GE was based on elevated glycine concentrations in blood and CSF with an elevated CSF:plasma glycine ratio, and normal organic acids in urine. Diagnosis was confirmed by enzyme assay in 13 patients performed by Dr. Rolland, Lyon, France, or Prof. Applegarth, Vancouver, Canada, and by mutation analysis in 14 patients.

Classification

Patients were classified according to developmental outcome as severe (IQ <20) or attenuated (IQ >20). They were also classified according to age of onset as neonatal (<1 week of age) or infantile (>1 week of age). Thus, in combination we observed four different groups: Group I: *severe neonatal GE:* children with onset during the first week of life and poor long-term outcome (IQ<20); group II: *attenuated neonatal GE:* children with onset during the first week of life but better long-term outcome (IQ>20); group III: *severe infantile GE*: children with onset after the neonatal period and poor long-term outcome (IQ<20); and group IV: *attenuated infantile GE:* children with onset after the neonatal period and better long-term outcome (IQ>20). Based on their neurological outcome groups I and III were summarized as *severe GE*, groups II and IV as *attenuated GE* (Table 1).

Data

We collected data on pregnancy, perinatal period, initial clinical symptoms, initial laboratory findings, age at onset of symptoms, clinical course, and developmental outcome.

Table 1 Classification of different forms of GE

GE form	Age at manifestation	Number of patients	Outcome	Classification due to outcome	Number of patients
Severe neonatal GE	First week of life	33	IQ<20	Severe GE	36
Severe infantile GE	Onset after neonatal period	3			
Attenuated neonatal GE	First week of life	6	IQ>20	Attenuated GE	9
Attenuated infantile GE	Onset after neonatal period	3			

Data on EEG and cerebral imaging (ultrasound, CT, MRI) were reviewed. We compared therapies and their effect on laboratory data and clinical outcome. Informed consent was obtained from all parents.

Severity score system

Based on previously reported data, we developed a severity score to predict the long-term outcome in patients with GE (Hennermann 2006). This severity score was determined using the following parameters at age 12 months: age at onset ≤3 months of age (+1), intractable seizures (+1), EEG burst suppression pattern (+1), EEG hypsarrhythmia pattern (+1), hypoplasia of the corpus callosum (+1), other cerebral malformations (+1), further congenital malformations (+1), start of spasticity before age of 6 months (+1), frequent hiccuping (+1), feeding difficulties (+1), hyperactivtiy (−1), and choreiform movement disorders (−1). For determining outcome, the following parameters were used at age >18 months: smiling (+1), head control (+1), grasp (+1), sit (+1), walk (+1), and speak (+1). This score was only applied in patients in whom at least 11/12 parameters for the severity score, and at least 5/6 parameters for the outcome were available (n=35).

Statistic analyses

For statistic analyses PASW Statistics, Version 18.0, was used performing one sample T test, and Spearman's rho correlation coefficient. For graphic presentation bivariate scatterplots were used.

Results

Subjects

There were 36 children with severe GE (33 severe neonatal GE, three severe infantile GE), and nine children with attenuated GE (six attenuated neonatal GE, three attenuated infantile GE) (Table 1). Of children with severe GE 19/36 died at a median age of 31.5 months (3 days to 17.3 years). Five of them died during the neonatal period. Due to their severe clinical course, the occurrence of malformations, and severely affected siblings, they were classified as severe GE. Only 1/9 patients with attenuated GE died (at age 6.9 years). At the time of examination, patients were aged 3 days to 26.1 years (median 4.6 years). Living patients were aged 4 months to 26.1 years (median 4.6 years). The oldest patient was a 26 year old woman, to our knowledge the oldest reported patient with severe GE.

Pre- and perinatal complications

Pregnancy was complicated in seven children with GE by maternal bleeding, arterial hypertension, oligohydramnios, or gestational diabetes. Prematurity was not increased (total: 9%). Seven children (16%) with severe GE were born small for date.

Age and clinical symptoms at presentation

All children with neonatal onset of GE presented within the first three days of life. Of infantile GE, children with the severe infantile GE presented earlier (median age 8.5 weeks, range 3–9 weeks) than children with attenuated infantile GE (median age 8.75 months, range 5–12 months). Hypotonia was the initial cardinal symptom in all GE subtypes. The next most common presenting symptoms were seizures, coma, and apnea. Apnea requiring intubation only occurred in children with neonatal onset and did not differentiate between severe and attenuated forms (Table 2).

Initial laboratory findings and confirmation of diagnosis

At diagnosis, the highest plasma and CSF glycine concentrations (Table 3) were found in patients with neonatal onset of GE. Glycine in CSF decreased with age and was significantly higher in patients with neonatal onset than in infantile onset unrelated to outcome. In children with severe neonatal GE, initial CSF:plasma glycine ratio was >0.07. A CSF:plasma ratio of ≤0.07 was usually associated with a better outcome (Dinopoulos et al. 2005), but a CSF:plasma ratio ≥0.08 did not discriminate between the different GE forms. One severe neonatal GE patient had an atypically

Table 2 Clinical symptoms in different forms of GE

Clinical symptoms	Severe neonatal GE	Attenuated neonatal GE	Severe infantile GE	Attenuated infantile GE	Total	Percent
Hypotonia (at presentation)[1]	33/33	6/6	3/3	3/3	45/45	100%
Seizures (at presentation)[1,2]	28/33	4/6	3/3	3/3	38/45	84%
Coma (at presentation)[1]	25/33	5/6	2/3	1/3	33/45	73%
Apnea (at presentation)[1]	26/33	5/6	0/3	0/3	31/45	67%
Seizures (on long-term)	28/28	4/6	3/3	3/3	38/40	95%
Spasticity[1]	26/26	3/6	3/3	0/3	32/38	84%
Spasticity before age of 6 months[3]	25/26	0/6	0/3	0/3	25/38	66%
Hiccups[3]	21/22	2/6	2/2	0/3	25/34	74%
Microcephaly	22/28	3/6	2/3	0/3	27/40	68%
Hyperactivity	6/27	6/6	2/3	3/3	17/39	44%
Choreiform movement disorder	5/27	5/6	0/3	3/3	13/39	33%
Macrocephaly[4]	3/28	0/6	0/3	0/3	3/40	8%
Burst suppression pattern (EEG)[3]	25/31	3/5	1/3	0/3	29/42	69%
Hysarrhythmia pattern (EEG)[3]	16/23	0/5	3/3	1/3	20/34	59%

[1] Initial symptoms at time of presentation

[2] Seizures commenced after presentation in three children with severe neonatal GE (at age 28 days, 42 days, and 47 days respectively) and in one child with attenuated neonatal GE (at age 2 months). Therefore, these data were not included with the initial clinical symptoms

[3] Data were not reported for every patient

[4] Macrocephaly was directly associated with hydrocephalus (see Table 5)

low CSF:plasma ratio (0.05) due to unusually high initial serum glycine. Insufficient data on GCS enzyme activities and molecular analyses in this study did not allow for a generalizable conclusion.

Short- and long-term problems

Spasticity occurred early in severe neonatal GE at the age of ≤3 months, in severe infantile GE at the age of 8–12 months, and in attenuated neonatal GE at the age of 2 years. None of the children with attenuated infantile GE developed spasticity. Seizures occurred in all forms of GE, but remained persistent and intractable only in severe GE. EEG patterns with hypsarrhythmia or burst-suppression-pattern were more frequent in severe GE (73% and 76%, respectively) than in attenuated GE (13% and 38%, respectively). Microcephaly (77% vs. 33%) and hiccups (96% vs. 22%) were more frequent in severe GE. Almost all children with attenuated GE developed severe hyperactivity and choreiform movements (89% in attenuated GE vs. 17% in severe GE). Details on the clinical presentation are shown in Table 2. Children with severe GE had more gastrointestinal problems: feeding difficulties requiring gastric tube feeding (61% in severe vs. 33% in attenuated),

Table 3 Initial laboratory findings in different forms of GE

Laboratory data	Severe neonatal GE	Attenuated neonatal GE	Severe infantile GE	Attenuated infantile GE	Healthy newborn (1–28 days)	Healthy infant (1 – 12 months)
Glycine in plasma (μmol/L)	1360±788** (420–4090)	1232±786** (680–2800)	751±360* (340–1020)	929±250** (640–1080)	232–740	81–436
Glycine in CSF (μmol/L)	216±252** (40–1440)	228±180** (70–570)	102±42* (60–140)	85±27** (50–100)	3–23[2]	3–23[2]
CSF:plasma ratio	0.18±0.08** (0.05–0.43) [1]	0.20±0.05* (0.13–0.26)	0.20±0.19* (0.07–0.42)	0.10±0.05* (0.05–0.16)	0.012–0.04	0.012–0.04

Values are given as mean ± SD (minimum and maximum value); * not significant, ** significant

[1] In one patient with severe neonatal GE initial serum glycine was extremely high (4086 μmol/L), which despite high CSF glycine (222 μmol/L) resulted in an atypical low CSF:plasma ratio (0.05). In all other children with severe neonatal GE initial CSF:plasma ratio was >0.07

[2]Derived from Jones et al. 2006

gastroesophageal reflux with esophagitis (35% vs. 11%), and bile stones (6% vs. 0%).

Mental outcome

Only one fourth of children with severe GE learned to smile, and, except for three of them, they never learned to grasp or to sit. In attenuated GE, mental outcome was better, but still poor. Of the 37 patients that survived sufficiently long to evaluate their developmental outcome, 13 learned to grasp, ten learned to sit, nine learned to walk and to babble, and five children learned to speak at least some words. Patients presenting in infancy (50%) had a better chance of making developmental progress than children presenting neonatally (19%) (Table 4). Children with attenuated neonatal GE learned to sit at the age of 7–40 months and to walk at the age of 17–120 months; children with attenuated infantile GE learned to sit at the age of 14–18 months and to walk at the age of 18–39 months. Speech development was severely delayed in all GE forms. Even children with attenuated GE only learned to babble or at best to speak some words. There was no significant gender difference in outcome (Supplementary Table).

Birth defects including cerebral malformations and abnormalities

Malformations were only present in children with severe neonatal GE: three showed club feet, two unilateral ptosis, one micrognathia and dysplastic ears, one a hemangioma localized in the liver, three congenital hernias, and seven boys with cryptorchism.

On cerebral imaging, hypoplasia of the corpus callosum was the most frequent abnormality (50% of all children with GE). This malformation was only found in severe GE, was already present neonatally and persisted. Similarly, other cerebral abnormalities, e.g. brain atrophy, hydrocephalus, or posterior fossa cyst, only occurred in severe neonatal GE (Table 5).

Score system

Severity score was significantly higher in children with severe GE (median +7, range 4–10) than in those with attenuated GE (median −1, range −2 - 1). The outcome score was significantly lower in children with severe GE (median ±0, range 0–3) than in those with attenuated GE (median +6, range 6–6). There was a significant negative correlation between severity score and neurodevelopmental outcome ($p=0.000$, $r^2=0.873$) (Fig. 1). There were no significant gender differences, either in severity score or outcome.

Treatment

A positive biochemical effect of treatment was defined as a reduction in plasma glycine to ≤300 μmol/L. A positive clinical effect was defined as an increase in alertness, a decrease of seizures, or a decreased number of anticonvulsants. Comparing treatment effectiveness in different GE forms, benzoate showed most frequently a positive effect on both initial and long-term treatment in all GE forms. In children in whom benzoate treatment was not effective, benzoate doses may have been far too low (Table 6). Benzoate treatment was limited by poor compliance due to its unpalatability and its gastrointestinal side effects, by benzoate intoxication in one single patient (Van Hove et al. 2005), and by carnitine depletion in five patients. A protein restricted diet improved glycine reduction in a few children, independent of the form of GE. Side effects of diet were poor compliance, protein deficiency in two, and vitamin B_{12} deficiency in one child.

Dextromethorphan therapy showed initially a good response in all GE forms, but was effective for long-term treatment mostly in children with attenuated GE. During the

Table 4 Neurodevelopmental outcome in children with GE

Development	Severe neonatal GE	Attenuated neonatal GE	Severe infantile GE	Attenuated infantile GE	Total
Deceased	17/33	0/6	2/3	1/3	16/40
Median age of death (months)	48	-	96	72	50
Smile[1]	6/25	6/6	2/3	3/3	17/37
Grasp[1]	3/25	6/6	1/3	3/3	13/37
Sit alone[1]	1/25	6/6	0/3	3/3	10/37
Walk alone[1]	0/25	6/6[2]	0/3	3/3	9/37
Babble[1]	0/25	6/6	0/3	3/3	9/37
Speak words[1]	0/25	2/6	0/3	3/3	5/37

[1]Long-term outcome was only evaluated in children surviving the neonatal period and the first 15 months of life

[2]Walking with support

Table 5 Cerebral imaging in different forms of GE

Changes in cerebral imaging	Severe neonatal GE[1]	Attenuated neonatal GE[2]	Severe infantile GE[2]	Attenuated infantile GE[2]	Total	Percent
Hypoplasia of the corpus callosum	18/29	0/6	1/3	0/3	19/41	46%
Enlarged ventricles[3]	12/29	0/6	1/3	2/3	15/41	37%
Delayed myelination[3]	8/20	0/6	2/3	0/3	10/32	31%
Hyperintensity of the periventricular white matter and internal capsule [on T2]	6/17	0/6	0/3	1/3	7/29	24%
Brain atrophy[3]	8/29	0/6	0/3	0/3	8/41	20%
Posterior fossa cyst[3]	5/29	0/6	0/3	0/3	5/41	12%
Hydrocephalus	3/29	0/6	0/3	0/3	3/41	7%
Macrogyria	3/29	0/6	0/3	0/3	3/41	7%
Cerebellar hypoplasia[3]	2/29	0/6	0/3	0/3	2/41	5%
Necrotizing cerebral areas	2/29	0/6	0/3	0/3	2/41	5%

[1] Ultrasound was performed in 15 children, CT in three children and MRI in 18 children, four children were not examined

[2] MRI was performed in all children with attenuated neonatal, severe infantile and attenuated infantile GE

[3] In two children ultrasound performed in neonatal period revealed no abnormalities. Changes were found first at the age of one month and 6 years

neonatal period, treatment had been withdrawn in three patients; all of them had affected sibs with severe GE.

During long-term treatment, the number of anticonvulsants used in patients with severe GE had to be increased to as much as four, whereas in attenuated GE no or only one anticonvulsant was used. Phenobarbital was mostly effective during the neonatal period (18/34 treated patients). Seizures partly improved by treatment with primidone (4/5 treated patients), levetiracetam (2/3), phenytoin (2/9), vigabatrin (3/14), topiramate (1/4), carbamazepine (1/5), and clobazam (1/6). Lamotrigine and sultiame were not effective. Valproate improved seizures in one child with severe neonatal GE, but was associated with severe side effects in four children. Three children received felbamate without effect on short-term treatment in two, but with a dramatic improvement in one child with therapy-resistant seizures. Strychnine was effective in apneic neonates resulting in extubation. Baclofen only slightly improved spasticity. Imipramine, promethazine, and neuroleptic drugs had little or even a paradoxical effect on hyperactivity.

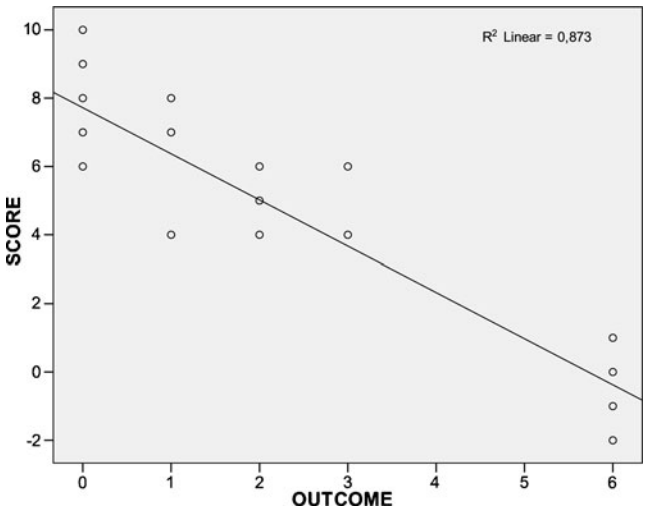

Fig. 1 The relationship between the severity score and neurodevelopmental outcome in patients with GE. The severity score was calculated on the presence of typical clinical symptoms in children with GE. A high severity score was associated with a high frequency of these clinical symptoms, a low severity score with a low frequency. Outcome was defined by reaching certain milestones of development. A high outcome score reflected better development, a low outcome score poor development. The correlation between the severity score and the outcome score was significant ($p<10^{-3}$)

Discussion

Although GE is one of the more frequent neurotransmitter disorders, only a few clinical surveys have been performed (Hoover-Fong et al. 2004; Carson 1982). In this systematic study of clinical data on 45 patients with different forms of GE, we identified parameters and a severity score that allows a prediction of the long-term outcome in infancy. While patients who present in the neonatal period have a higher likelihood of poor outcome than patients presenting in infancy, there are many exceptions. 19% of children presenting in the newborn period and 50% of children presenting in infancy made developmental progress, a frequency consistent with the previous survey (Hoover-Fong et al. 2004). This information is essential for correct counseling in the neonatal period about the likelihood of developmental progress. Whereas children making developmental progress are usually still substantially impaired, parents find this distinction substantive and important. This

Table 6 Effect of treatment in different forms of GE

Treatment		Severe neonatal GE	Attenuated neonatal GE	Severe infantile GE	Attenuated infantile GE
Total number		28	6	3	3
Benzoate[1]	on treatment	26	6	3	3
	dosage (mg/kg/day)	350 (30–750)	310 (200–425)	575 (235–800)	450
	positive effect	22	6	2	1
Dextromethorphan[2]	on treatment	13 (11)	2	2	3
	dosage (mg/kg/day)	7.5 (2.15–30)	4.1 (3.2–5)	25.2 (5.4–45)	7.5 (6.3–8)
	positive effect	8 (3)	2	1	3
Strychnine[3]	on treatment	6	2	0	0
	positive effect	4	2	0	0
Number of anticonvulsants (on long-term treatment)		2 - 4	0 - 1	1 - 3	0 - 1
On diet		17	3	2	0

[1] In children with no effect of benzoate treatment the daily dose was less than 290 mg/kg

[2] Long-term treatment and long-term effect of dextromethorphan treatment in severe neonatal GE is indicated in brackets

[3] Strychnine was only applied during the neonatal period (dosage 0.1–1.9 mg/kg/day)

study did not include children presenting at >1 year of age, in which even near normal outcome has been reported (Brunel-Guitton et al. 2011).

Initial symptoms consisted of hypotonia, seizures, coma, and apnea. Their occurrence was associated with the age at manifestation, rather than with the final outcome. Older children less often showed coma or apnea, regardless of their long-term outcome. Some patients with severe neonatal GE initially presented without overt clinical seizures, but only with hypotonia and coma. Certain clinical parameters predict more reliably the long-term outcome: spasticity developing within the first six months of life, frequent hiccupping, and microcephaly are associated with a poor outcome, whereas hyperactivity and choreiform movements are associated with attenuated GE (Dinopoulos et al. 2005). Typical EEG patterns, burst-suppression in neonates and hypsarrhythmia in infants, are often, but not always, associated with a poor outcome. Our data do not reveal a gender difference in mortality and mental outcome, in contrast to a previous survey (Hoover-Fong et al. 2004).

Our data confirm that severe cerebral malformations, including hypoplasia of the corpus callosum, always predict a very poor outcome (Hoover-Fong et al. 2004; Van Hove et al. 2000; Fletcher et al. 1995; Yis et al. 2009). Extracerebral congenital malformations, e.g. club feet, hernias, and cryptorchism reflecting intrauterine hypotonia predict a severe outcome as well. This likely represents a damaging effect of high glycine levels in utero.

The ethical decision making for GE in the neonatal period has been reviewed (Boneh et al. 2008). Limited data are available to discriminate outcome at this stage. Only cerebral malformations on MRI have absolute prognostic value, whereas certain signs such as burst suppression pattern are only partially predictive. The identification of mild mutations associated with attenuated neonatal GE (Kure et al. 2004) requires more time than the decision making allows for. To improve counseling and decision making on treatment in infants with GE we developed a new severity score which predicts outcome in infancy. This score reveals a significant correlation between selected clinical parameters and the long-term outcome of the disease. Therefore its application may ease medical management. As the occurrence of cerebral malformations is important for determining the severity score, early cerebral brain imaging with MRI is extremely useful in predicting disease severity.

The CSF:plasma glycine ratio has been suggested to distinguish GE forms (Hamosh and Johnston 2001), and to relate to severity (Boneh et al. 2008). This assumption was not confirmed by our data, unless at the extreme, and has been discussed before (Boneh et al. 1996). Although severe GE is often associated with very high glycine plasma concentrations, initial low plasma glycine levels do not exclude a severe GE form. The age dependency of the CSF glycine concentrations and the CSF:plasma ratio make interpretation for severity prediction difficult. Specific mutations, such as p. A802V, have been suggested to predict an outcome of the attenuated form even in patients with a neonatal presentation, which emphasizes the impact of treatment in the neonatal period (Korman et al. 2004; Kure et al. 2004; Brunel-Guitton et al. 2011). Insufficient data are available to confirm this in the data of the present study.

As already shown in previous studies (Hoover-Fong et al. 2004; Wolff et al. 1986; Van Hove et al. 1995), most effective was the treatment with sodium benzoate. However, the effect of benzoate was dependent on the dose and

on the severity of the disease. The dose required to normalize plasma glycine levels is different in severe and attenuated forms of GE (Van Hove et al. 2005). As our and previous data show, side effects of benzoate treatment may be avoided by application of saliva resistant coated benzoate granules (Breitkreutz et al. 2003a, b) and by regular measurement of glycine, benzoate, and carnitine plasma levels (Van Hove et al. 1995; Wolff et al. 1986; Van Hove et al. 2005). Dextromethorphan, an NMDA receptor antagonist, decreased seizures in children with GE in our and previous studies (Hamosh et al. 1992; Schmitt et al. 1993). Our data now indicate that dextromethorphan is more effective during long-term treatment in attenuated than in severe GE. Strychnine improved respiration during the neonatal period in all GE forms, but its long-term use has deleterious effects (Hamosh and Johnston 2001).

The treatment of convulsions in GE is challenging, and patients with severe GE require multiple medications. While our study was not designed to provide a controlled analysis of the efficacy of various antiepileptics, certain preliminary conclusions can be drawn from the experience in our study. Seizure improvement was noted with primidone, levetiracetam, phenytoin, vigabatrin, topiramate, carbamazepine, and clobazam. Phenobarbital and benzodiazepines were mostly effective in early infancy. Felbamate, a NMDA-receptor-blocker (Harty and Rogawski 2000), can be considered for the treatment of recalcitrant seizures, although serious side effects limit its wider use. Certain anticonvulsants such as lamotrigine and sultiame only rarely improved seizures in GE. Valproate inhibits residual enzyme activity and was associated with severe side effects in attenuated GE (Hoover-Fong et al. 2004; Hall and Ringel 2004; Morrison et al. 2006). These findings can be used to guide the choice of anticonvulsants, but as our data are limited, further studies are necessary to evaluate the most effective approach for seizure control.

In conclusion, our data indicate that the outcome of GE can be predicted by scoring selected clinical parameters. Early cerebral brain imaging is extremely useful in predicting the severity of the disease.

Acknowledgement We thank all the children and their parents for participation and for their cooperation.

Special thanks to the cooperating colleagues G. Froese, Böblingen, Germany; S. Stolz, Cottbus, Germany; U. Spiekerkötter and U. Wendel, Düsseldorf, Germany; I. Knerr, Erlangen, Germany; J. Herwig, Frankfurt, Germany; W. Lehnert, Freiburg, Germany; J. Kreuder, Giessen, Germany; G. F. Hoffmann, Heidelberg, Germany; T. Marquardt, Münster, Germany; R. Ensenauer, Munich, Germany; F. K. Trefz and H. Korall, Reutlingen, Germany; F. Walter, Rostock, Germany; M. Häussler, Würzburg, Germany; C. Nuttin, Luxembourg; J Smeitink and R Sengers, Nijmegen, The Netherlands; J. Jaeken, Leuven, Belgium; and the general practitioners.

We thank Dr. Janet Thomas, MD for her careful review of the manuscript and constructive comments.

We thank Nutricia/SHS, Heilbronn, Germany, for support of J-M Berger. JLK Van Hove and G Scharer are supported by the NKH Crusader Fund and the Hope for NKH Fund.

Disclosure Travelling expenses for J-M Berger were overtaken by Nutricia/SHS, Heilbronn, Germany.

References

Applegarth DA, Toone JR (2001) Nonketotic hyperglycinemia (glycine encephalopathy): laboratory diagnosis. Mol Genet Metab 74:139–146

Applegarth DA, Toone JR (2004) Workshop report. Glycine encephalopathy (nonketotic hyperglycinemia): review and update. J Inherit Metab Dis 27:417–422

Applegarth DA, Toone JR, Lowry RB (2000) Incidence of inborn errors of metabolism in British Columbia, 1969–1996. Pediatrics 105:e10

Boneh A, Degani Y, Harari M (1996) Prognostic clues and outcome of early treatment of nonketotic hyperglycinemia. Pediatr Neurol 15:137–141

Boneh A, Allan S, Mendelson D, Spriggs M, Gillam LH, Korman SH (2008) Clinical, ethical, and legal considerations in the treatment of newborns with non-ketotic hyperglycinemia. Mol Genet Metab 94:143–147

Breitkreutz J, Bornhöft M, Wöll F, Kleinebudde P (2003a) Pediatric drug formulations of sodium benzoate: I. Coated granules with a hydrophilic binder. Eur J Pharm Biopharm 56:247–253

Breitkreutz J, El-Saleh F, Kiera C, Kleinebudde P, Wiedey W (2003b) Pediatric drug formulations of sodium benzoate: II. Coated granules with a lipophilic binder. Eur J Pharm Biopharm 56:255–260

Brunel-Guitton C, Casey B, Coulter-Mackie M et al. (2011) Late-onset nonketotic hyperglycinemia caused by a novel homozygous missense mutation in the *GLDC* gene. Mol Genet Metab 103:193–196

Busanello EN, Moura AP, Viegas CM et al. (2010) Neurochemical evidence that glycine induces bioenergetical dysfunction. Neurochem Int 56:948–954

Carson NAJ (1982) Non-ketotic hyperglycinemia – a review of 70 patients. J Inheret Metab Dis 5(Suppl 2):126–128

Dinopoulos A, Matsubara Y, Kure S (2005) Atypical variants of nonketotic hyperglycinemia. Mol Genet Metab 86:61–69

Fletcher JM, Bye AM, Nayannar V, Wilcken B (1995) Non-ketotic hyperglycinaemia presenting as pachygyria. J Inherit Metab Dis 18:665–668

Hall DA, Ringel SP (2004) Adult nonketotic hyperglycinemia (NKH) crisis presenting as severe chorea and encephalopathy. Mov Disord 19:485–486

Hamosh A, Johnston MV (2001) The metabolic and molecular bases of inherited disease. In: Sciver CR, Beaudet AL, Sly WS, Valle D (eds) Nonketotic hyperglycinemia, 8th edn. McGraw-Hill, New York, pp 2065–2078

Hamosh A, Mcdonald JW, Valle D, Francomano CA, Niedermeyer E, Johnston MV (1992) Dextromethorphan and high-dose benzoate therapy for nonketotic hyperglycinemia in an infant. J Pediatr 132:709–713

Harty TP, Rogawski MA (2000) Felbamate block of recombinant N-methyl-D-aspartate receptors: selectivity for the NR2B subunit. Epilepsy Res 39:47–55

Hennermann JB (2006) Clinical variability in glycine encephalopathy. Future Neurol 1:621–630

Hoover-Fong JE, Shah S, Van Hove JLK, Applegarth D, Toone J, Hamosh A (2004) Natural history of nonketotic hyperglycinemia in 65 patients. Neurology 63:1847–1853

Jones CM, Smith M, Henderson MJ (2006) Reference data for cerebrospinal fluid and the utility of amino acid measurement for the diagnosis of inborn errors of metabolism. Ann Clin Biochem 43:63–66

Korman SH, Boneh A, Ichinohe A, Kojima K, Sato K, Ergaz Z, Gomori JM, Gutman A, Kure S (2004) Persistent NKH with transient or absent symptoms and a homozygous GLDC mutation. Ann Neurol 56:139–143

Kure S, Ichinohe A, Kojima K, Sato K, Kizaki Z, Inoue F, Yamanaka C, Matsubara Y (2004) Mild variant of nonketotic hyperglycinemia with typical neonatal presentations: mutational and in vitro expression analyses in two patients. J Pediatr 144:827–829

Morrison PF, Sankar R, Shields WD (2006) Valproate-induced chorea and encephalopathy in atypical nonketotic hyperglycinemia. Pediatr Neurol 35:356–358

Nguyen L, Rigo J-M, Rocher V et al. (2001) Neurotransmitters as early signal for central nervous system development. Cell Tissue Res 305:187–202

Schmitt B, Steinmann B, Gitzelmann R, Thun-Hohenstein L, Mascher H, Dumermuth G (1993) Nonketotic hyperglycinemia: clinical and electrophysiologic effects of dextromethorphan, an antagonist of the NMDA receptor. Neurology 43:421–424

Tada K, Kure S (1993) Non-ketotic hyperglycinemia: molecular lesion, diagnosis, and pathophysiology. J Inherit Metab Dis 16:691–703

Van Hove JLK, Kishnani P, Muenzer J et al. (1995) Benzoate therapy and carnitine deficiency in non-ketotic hyperglycinemia. Am J Med Genet 59:444–453

Van Hove JLK, Kishnani PS, Demaerel P et al. (2000) Acute hydrocephalus in nonketotic hyperglycinemia. Neurology 54:754–756

Van Hove JLK, Vande Kerckhove K, Hennermann JB et al. (2005) Benzoate treatment and the glycine index in nonketotic hyperglycinemia. J Inherit Metab Dis 28:651–663

Wolff JA, Kulovich S, Yu AL, Qiao CN, Nyhan WL (1986) The effectiveness of benzoate in the management of seizures in nonketotic hyperglycinemia. Am J Dis Child 140:596–602

Yis U, Kurul SH, Dirik E (2009) Nonketotic hyperglycinemia and acquired hydrocephalus. Pediatr Neurol 40:138–140