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Decline of Acute Encephalopathic Crises in Children with Glutaryl-CoA Dehydrogenase Deficiency Identified by Newborn Screening in Germany

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ABSTRACT: Glutaryl-CoA dehydrogenase (GCDH) deficiency is a rare neurometabolic disorder that is considered treatable if patients are identified before the onset of acute encephalopathic crises. To allow early identification of affected individuals, tandem mass spectrometry-based newborn screening for GCDH deficiency has been started in Germany in 1999. We prospectively followed neonatally screened patients ($n = 38$) and compared the neurologic outcome with patients from a historical cohort ($n = 62$). In the majority of neonatally screened children, the onset of encephalopathic crises has been prevented (89%), whereas acute encephalopathic crises or progressive neurologic impairment was common in the historical cohort. Neonatal screening in combination with intensive management is effective – even assuming ascertainment bias in the historical cohort. Similar proportions of commonest mutations and biochemical phenotypes (high and low excretors) were found in neonatally screened and historical patients. However, potential predictor variables for mild clinical phenotypes are not yet known and thus a selection of these patients by newborn screening is not excluded. No patient was known to be missed by newborn screening from 1999 to 2005. In conclusion, this study confirms that newborn screening for GCDH deficiency in combination with intensive management is beneficial. **(*Pediatr Res* 62: 357–363, 2007)**

Glutaryl-CoA dehydrogenase (GCDH; EC 1.3.99.7) deficiency (glutaric aciduria type I) is a "cerebral" organic acid disorder first described in 1975 (1). The estimated overall prevalence is 1 in 100,000 newborns (2), but is considerably higher in some genetic isolates (3,4). More than 150 disease-causing mutations have been identified (5,6). GCDH is a mitochondrial key enzyme in the final degradative pathways of L-lysine, L-hydroxylysine, and L-tryptophan (7). The prognostically relevant event is the onset of acute encephalopathic crises which are usually precipitated by episodes that are likely to induce catabolic state (*e.g.*, febrile illness) during a vulnerable period in infancy resulting in irreversible striatal injury and, subsequently, movement disorders (4,8,9). Life expectancy is strongly reduced in symptomatic patients (10,11). In two variant forms of GCDH deficiency (insidious onset, late onset), neurologic disease develops without overt crises, suggesting chronic neurodegenerative changes. If treatment starts before the onset of irreversible neurologic symptoms, encephalopathic crises can be prevented in the majority of patients (4,8,11–13).

The initial presentation of affected children is nonspecific, and macrocephaly is most frequently found (9). Therefore, metabolic screening techniques have been developed. The key metabolites can be detected by gas chromatography/mass spectrometry (GC/MS) of glutaric acid (GA) and 3-hydroxyglutaric acid (3-OH-GA; ref. 14) or tandem mass spectrometry (MS/MS) of glutarylcarnitine (C5DC; refs. 2,15). MS/MS-based newborn screening (NBS) programs have been established in some countries (15–17). In Germany, MS/MS-based NBS has been initiated in 1999 in some federal countries (Baden-Württemberg, Bavaria, Lower Saxony) and, recently, it has been implemented into the nationwide NBS. The major aims of the present study were to investigate whether implementation of NBS for GCDH deficiency and intensive management influences the disease course and neurologic outcome of affected individuals.

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Abbreviations: 3-OH-GA, 3-hydroxyglutaric acid; **C5DC,** glutarylcarnitine; **GA,** glutaric acid; **GCDH,** glutaryl-CoA dehydrogenase (EC 1.3.99.7); **GC/MS,** gas chromatography/mass spectrometry; **MS/MS,** tandem mass spectrometry; **NBS,** newborn screening

PATIENTS AND METHODS

Study population. We studied 100 patients (44 female, 56 male) with GCDH deficiency. Diagnosis was confirmed by analysis of *GCDH* gene mutations ($n = 82$) and/or enzymatic analysis of GCDH activity ($n = 70$; Prof. Christensen, Copenhagen, Denmark). Patients were anonymized by initials, birthdates, and gender, and double entries were excluded by these parameters. Data were only included if written informed consent was obtained from patients and/or parents. The study was approved by the Institutional Ethics Committee of the University of Heidelberg (# 314/2002).

Patient groups and study protocol. From 1999–2005, NBS in Germany identified 38 patients with GCDH deficiency ("NBS group"). Prospective follow-up was performed using a standardized protocol (URL: *www.metab-net.de/index.php?lang = en&ID = 4&sID = 14&psID = 4*; subproject "Glutaric aciduria type I", Link: "Instruments") for therapy and therapy monitoring (18). Briefly, for maintenance treatment a combination of dietary treatment (lysine restriction plus lysine-free, tryptophan-reduced amino acids supplements) and L-carnitine supplementation was used. Treatment was intensified during episodes that are likely to precipitate encephalopathic crises, *e.g.*, febrile illness. Stop of natural protein intake, administration of high-caloric fluids, increasing the carnitine dosage, and administration of antipyretics and/or antibiotics were transiently used during these episodes and maintenance treatment was re-introduced stepwise. Similar treatment protocols (including lysine-restricted diet) have been used at German metabolic centers since the early 1990s. In general, dietary treatment and pharmacotherapy was implemented by an interdisciplinary team including metabolic pediatricians, dieticians, nurses and occupational therapists, and parents were trained by this team.

In addition, 62 patients were identified before the start of NBS ("historical patients"). In analogy to a previous study (11), data from this cohort of patients were collected by a standardized questionnaire.

Statistical analysis. The cut-off date for statistical analysis was October 31, 2006. We used nonparametric tests to compare outcome variables, and Kaplan-Meier analysis to calculate survival and encephalopathic crises. SD scores of anthropometrical data were calculated (19) using LMS values for body mass index (20), length and weight (kindly provided by Dr. K. Kromeyer-Hauschild), and head circumference (21).

We calculated the prevalence of GCDH deficiency in three different birth cohorts, *i.e.*, cohort I (year of birth 1975–1990), cohort II (1991–1998), and cohort III (1999–2005). This division was made to mark important 'milestones' in the medical history of this disease, including the first description in 1975 (1), publication of the first major case series in 1991 (22–24), and the start of NBS in Germany in 1999. Numbers for annual birth rates in Germany were obtained from the *Statistisches Bundesamt Deutschland* (URL: www.destatis.de) and numbers of screened neonates were kindly provided by the *Deutsche Gesellschaft für Neugeborenenscreening* (URL: www.neoscreening.de). Statistical analysis was performed using R (25).

RESULTS

Study population. One hundred patients with confirmed diagnosis of GCDH deficiency were included into this study. Patients were identified presymptomatically by NBS ($n = 38$), or due to high-risk screening of macrocephalic infants ($n = 4$) or siblings of previously identified patients ($n = 5$), whereas 53 patients were diagnosed by selective screening after the onset of neurologic symptoms. The majority of patients was of German origin (59/100), whereas 41 patients were from migrating families, mostly of Turkish ancestry. The cumulative follow-up time of all study patients was 685 y. The median age at latest report in the NBS group was 49 mo (range: 10–196 mo) and 133 mo (range: 5–826 mo) in historical patients. Diagnosis was made at a median age of 0.25 mo in the NBS group and at 15 mo of age in historical patients.

Estimated prevalence in neonatally screened and historical patients. We calculated the prevalence of GCDH deficiency in three different birth cohorts, *i.e.*, cohort I (year of birth 1975–1990), cohort II (1991–1998), and cohort III (1999–2005). This division was made to mark important 'milestones' in the knowledge and awareness of this disease, including the first description in 1975 (1), publication of the first major case series in 1991 (22–24), and the start of NBS in Germany in 1999. The lowest prevalence (mean: 1:611,400 newborns; 95% CI: 1:925,800-403,800) was found in cohort I, whereas it markedly increased in cohorts II (mean: 1:187,200 newborns; 95% CI: 1:261,600-134,000) and III (mean: 1:100,200 newborns; 95% CI: 1:138,100-72,700). These results suggest that selective screening (cohorts I and II) is highly dependent on the awareness of physicians for GCDH deficiency and thus includes a high risk to miss patients. It is not unexpected that the highest mean prevalence was found in cohort III (NBS group), since only NBS allows reliable identification of these patients. However, the number of historical patients (cohorts I and II) who have died undiagnosed is unknown due to the lack of a national tracking system.

Disease course and survival: NBS versus historical patients. It has been demonstrated that the onset of encephalopathic crises is *the* prognostically relevant event in GCDH deficiency (4,8–11). These studies have highlighted that the majority of first crises occur by age 24 mo, which allowed estimating the disease course after a relatively short observation period. This was confirmed by the present study showing that 95% of first crises occurred by age 24 mo (median: 10 mo; range: 1–37 mo). Importantly, all study patients except three children from the NBS group were older than 24 mo.

Forty-six patients had encephalopathic crises; the majority (67%) had only a single crisis. Kaplan-Maier analysis (Fig. 1) showed that the frequency of encephalopathic crises was lower in the NBS group (4/38 patients) and in presymptomatically diagnosed patients from the historical cohort (1/9 patients) than in symptomatic patients from the historical

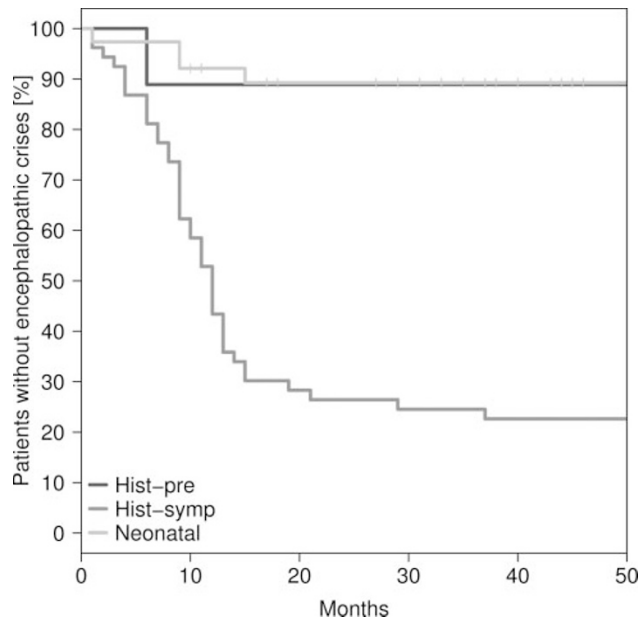

Figure 1. Encephalopathic crises in neonatally screened and historical patients. Kaplan-Meier analysis shows a lower frequency of encephalopathic crises (χ^2 (2) = 41.6, $p < 0.001$; log-rank test) in children identified presymptomatically by NBS ("neonatal"; $n = 38$) or in the historical cohort ("Hist-pre"; $n = 9$) than in historical patients identified after the onset of neurologic symptoms ("Hist-sympt"; $n = 53$). Most crises occurred by age 24 mo. No crisis has been reported after age 37 mo.

cohort (41/53 patients). The absolute risk reduction for the manifestation of encephalopathic crises between the NBS group (4/38 patients) and historical patients diagnosed after the onset of neurologic symptoms (41/53 patients) was 0.67, the number needed to treat to prevent one encephalopathic crisis was 1.50 confirming the efficacy of this management. In migrating families, however, neonatally screened children had more often crises (3/16 patients) than in families of German origin (1/22 patients).

In the four patients of the NBS group, who had had encephalopathic crises, start of emergency treatment was delayed for more than 24 h or even missed despite the onset of alarming symptoms, such as vomiting. In contrast, if emergency treatment was immediately started during threatening episodes ($n = 68$), no encephalopathic crisis occurred. Patients who had suffered encephalopathic crises developed dystonia (100%), and feeding problems (76%). Movement disorders were considered as severe (71%) or moderate (29%). As a consequence of severe neurologic symptoms, 11 children from the historical group died, whereas all presymptomatically diagnosed children have survived (Fig. 2). The most frequent known cause of death was pneumonia (36%). In contrast, all presymptomatically diagnosed children survived.

A subgroup of historical patients ($n = 15$) developed neurologic disease without known encephalopathic crises. Ten of these patients were classified as insidious-onset and 5 patients as late-onset type. In patients with insidious-onset type mild movement disorders (dystonia, ataxia) and learning difficulties were common, whereas patients classified as late-onset type presented with cephalgies, gait disturbance, slowing of fine motor function and tremor. The frequencies of disease courses in different patients groups are summarized in Fig. 3.

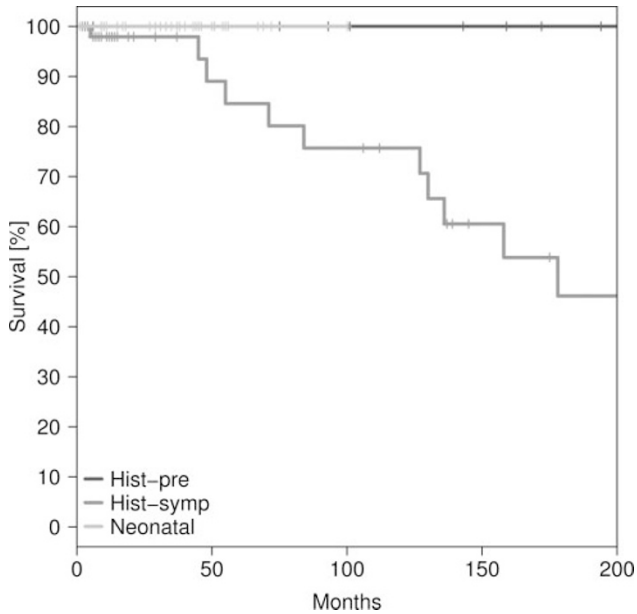

Figure 2. Survival rate in neonatally screened and historical patients. Kaplan-Maier analysis shows reduced life expectancy of symptomatic patients from the historical cohort ("Hist-symp"; $n = 53$) compared with children identified presymptomatically by NBS ("Neonatal"; $n = 38$) or in the historical cohort ("Hist-pre"; $n = 9$) who all have survived (χ^2 (2) = 6.5, $p < 0.05$; log-rank test). Note that the follow-up period in the NBS group is shorter than in the historical group.

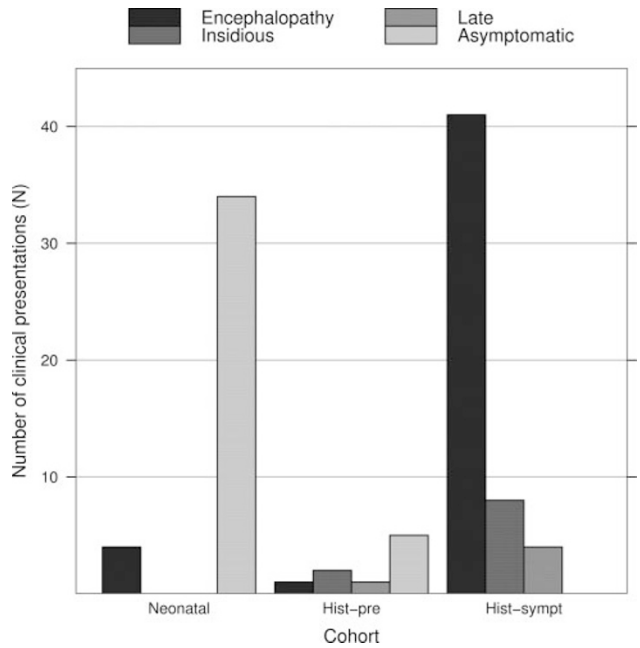

Figure 3. Clinical presentation in neonatally screened and historical patients. Neurologic symptoms were common in the majority of historical patients ("Hist-sympt"; $n = 53$), whereas only a few patients, who have been diagnosed presymptomatically ("Hist-pre"; $n = 9$), remained asymptomatic. Asymptomatic disease course was common in the NBS cohort. Encephalopathy, encephalopathic crises; insidious, insidious-onset type; late, late-onset type; asymptomatic, asymptomatic patients.

Gross motor development in the neonatal screening cohort. Since it has been reported that some infants with GCDH deficiency develop transient neurologic 'soft' signs (8), we investigated whether gross motor development was affected in neonatally screened infants. Transient trunkal hypotonia was found in 53% of infants. Between age 0 to 24 mo, most patients without an encephalopathic crisis ($n = 34$) reached six gross motor developmental milestones within the normal range according to the WHO motor development study (26), whereas some showed a mild to moderate delay (Fig. 4). In contrast, children with an encephalopathic crisis ($n = 4$) did not reach any of these motor milestones by age 24 mo.

Anthropometrics in the neonatal screening cohort. Presymptomatically diagnosed patients were treated by oral restriction of lysine intake plus lysine-free, tryptophan-reduced amino acids supplements, which also contained minerals and micronutrients, according to protocol for maintenance treatment (URL: *www.metabnet.de/index.php?lang = en&ID = 4&sID = 14&psID = 4*; subproject "Glutaric aciduria type I", Link: "Instruments"; 18). Since dietary restriction of natural protein includes a theoretical risk for malnutrition and failure to thrive, we investigated anthropometrical data in the NBS group by the age of 24 mo. Height, weight, and body mass index of these patients (Fig. 5A–C) were within the range expected in unaffected children (20). No patient was dystrophic, including four children who had had encephalopathic crises. Furthermore, an increased rate of obesity was not found in these children. As expected, the mean head circumference was increased owing to the disease-related high frequency of macrocephaly (Fig. 5D).

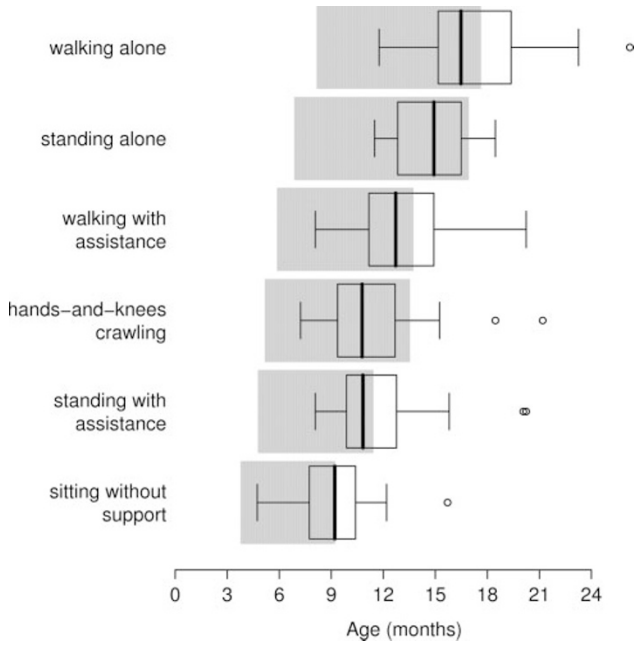

Figure 4. Gross motor development in the NBS group. Box plot diagram shows the achievement of six gross motor milestones in the NBS group from age 0 to 24 mo. Circles represent outliers and grey boxes indicate the windows of motor milestone achievement according to ref 25.

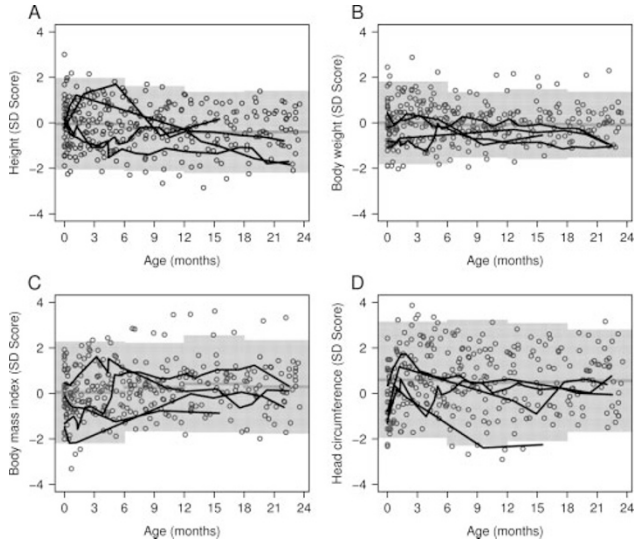

Figure 5. SD scores for height (A), weight (B), body mass index (C), and head circumference (D) in the NBS group. Grey boxes indicate the mean (grey lines) ±1.96 SD of measures within a 6-mo interval, whereas circles indicate single measures of asymptomatic patients. Black lines represent individuals with encephalophatic crises.

Genotypes and biochemical phenotypes. NBS versus historical patients. In some diseases, NBS has been shown to select patients with a mild biochemical phenotype. Therefore, we compared the genotypes and biochemical phenotypes in the NBS group and historical patients. First, *GCDH* gene analysis showed that the same mutations (E365K, P248L, R402W, A421V) were most frequently found in both groups (NBS group: 48%; historical patients: 55%). E365K and P248L were exclusively found in patients of Turkish origin and A421V in patients of German origin, whereas R402W, the most frequent mutation in Caucasians, was present in all ethnic groups. In line with mutation analysis, distribution of biochemical phenotypes was similar in both groups. According to the definition of Baric *et al.* (14), we found 82% high and 18% low excretors in the NBS group and 79% high and 21% low excretors in historical patients. Notably, NBS has reliably identified seven low excretors (Table 1), and, to our knowledge, no patient has been missed by NBS. GCDH analysis in fibroblasts demonstrated a median GCDH residual activity of 1% (range: 0–25%) in the NBS group and 0% (range: 0–30%) in historical patients. In conclusion, the distribution of genotypes and biochemical phenotypes in both subgroups were similar.

Table 1. *Characterization of low excretors identified by neonatal screening*

Patient no.	Allele 1	Allele 2	GCDH activity (% control)
1	R227P	R386X	Not determined
2	F236L	S259P	3
3	M1V	R227P	4
4	Y155H	A421V	5
5	S255W	Not found	6
6	G171W	V410M	8
7	R161Q	C228R	25

Comparison of siblings. There is no evidence that the genotype correlates with the clinical phenotype in GCDH deficiency owing to a high variation of the disease course among individuals (11). To investigate whether the disease course of affected siblings was influenced by the identification of index patients, we compared 24 siblings from 10 families (Table 2). Siblings shared the same *GCDH* gene mutations (not shown) and the same biochemical phenotype. The majority of index patients was identified after the onset of neurologic symptoms (8/10) at a median age of 19 mo, whereas only two index patients were identified presymptomatically due to macrocephaly (age at diagnosis: 1 mo, 24 mo). Three older siblings already had neurologic symptoms when being diagnosed. In contrast, the majority of younger siblings ($n = 11$), who were diagnosed presymptomatically, remained asymptomatic (9/11), whereas one suffered an encephalopathic crisis and one presented with insidious-onset type. These results underline that identification of presymptomatic patients is neuroprotective.

DISCUSSION

The major results of the present study are that 1) NBS in combination with early intensive management decreases the frequency of acute encephalopathic crises, 2) lysine-restricted dietary treatment supports normal growth and thrive, 3) the genotypes and biochemical phenotypes of patients from the NBS group and historical patient group are similar, and 4) NBS for GCDH deficiency is reliable for the identification of low excretors.

NBS and early intensive management are neuroprotective. Since the first report on GCDH deficiency 32 y ago (1) more than 400 patients have been identified worldwide (3,4,8–11,13). As a result of variations in the natural history, and differences in the diagnostic criteria and therapeutic protocols

Table 2. *Comparison of siblings*

Patient (No.)	Family	Gender	Age at diagnosis (Months)	Mode of diagnosis	Cause of selective screening	Encephalopathic crises	Disease course	Age at death (Months)	Excretor type
1*	A	M	24	Selective	Macrocephaly	—	Asymptomatic	—	High
2	A	M	1	HR	—	—	Asymptomatic	—	High
3*	B	M	37	Selective	PMR	—	Insidious	—	Low
4	B	M	0.25	NBS	—	—	Asymptomatic	—	Low
5*	C	M	1	Selective	Encephalopathy	+ (1 month)	Classical	5	High
6	C	F	1	HR	—	—	Asymptomatic	—	High
7*	D	F	4	Selective	Encephalopathy	+ (3 months)	Classical	—	High
8	D	M	0.25	NBS	—	—	Asymptomatic	—	High
9	D	F	0.25	NBS	—	—	Asymptomatic	—	High
10*	E	M	1	Selective	Macrocephaly	+ (6 months)	Classical	—	High
11	E	F	0.25	NBS	—	+ (9 months)	Classical	—	High
12*	F	M	25	Selective	PMR	—	Insidious	—	High
13	F	F	42	HR	—	—	Insidious	—	High
14	F	F	0.25	NBS	—	—	Asymptomatic	—	High
15*	G	M	96	Selective	Encephalopathy	+ (9 months)	Classical	—	High
16	G	M	111	HR	—	—	Insidious	—	High
17	G	F	47	HR	—	—	Asymptomatic	—	High
18	G	M	0.25	NBS	—	—	Asymptomatic	—	High
19*	H	M	8	Selective	Encephalopathy	+ (8 months)	Classical	—	High
20	H	F	53	HR	—	—	Insidious	—	High
21*	I	M	12	Selective	Encephalopathy	+ (12 months)	Classical	—	High
22	I	M	1	HR	—	—	Asymptomatic	—	High
23*	J	F	96	Selective	Encephalopathy	+ (6 months)	Classical	—	High
24	J	F	36	HR	—	—	Insidious	—	High

* Index patient; F, female; High, high excretor; HR, high-risk family screening (following identification of the index patient); Low, low excretor; M, male; NBS, newborn screening; PMR, psychomotor retardation; Selective, selective screening (following suggestive clinical presentation). Families D to H are related.

in different countries, the outcome has been varied. This has hampered our understanding of this disease and its treatability. Recently, we have demonstrated in 279 patients that presymptomatically diagnosed patients, who have received a combined maintenance therapy with lysine-restricted diet using natural protein with a low lysine content and amino acid supplementation (lysine-free, tryptophan-reduced) and oral carnitine supplementation, had the best outcome (11). However, the cross-sectional design of this study includes a moderate possibility for a confounder bias. For this purpose, we have conducted the present prospective follow-up study on children identified by neonatal screening in Germany ($n = 38$) and, subsequently, we compared outcome parameters with a historical patients group ($n = 62$).

The present study shows that the onset of prognostically relevant encephalopathic crises was markedly reduced (11% of patients) in the majority of children diagnosed by neonatal screening who have received intensive management. The same result was achieved in a small subgroup ($n = 9$) of historical patients diagnosed presymptomatically (11%). Most of these children achieved gross motor milestones without or with a mild delay, and dietary management did not affect growth and maturation. In contrast, historical patients, who already had symptoms when diagnosed, showed a high frequency of complications and reduced life expectancy owing to acute encephalopathic crises (76%) and variant disease forms, *i.e.*, insidious-onset (16%) and late-onset types (8%). Similar findings have been reported in a previous study (4), demonstrating that screening of asymptomatic newborns and, subsequently, careful follow-up management reduced the frequency of acute encephalopathic crises from 90% ($n = 57$) to 35% of patients ($n = 20$). Compared with this study, the frequency of acute crises was somewhat lower in our study, *i.e.*, 11% of neonatally diagnosed patients. Whether this discrepancy in the outcomes reflects differences in the natural history of included study patients, dietary treatment (no lysine-free, tryptophan-reduced amino acid supplements were used by Strauss *et al.* (4)), emergency treatment (in our study, intensified emergency treatment was usually performed at hospital), or environmental or socio-economic parameters is not yet known.

Does NBS for GCDH deficiency select patients with a mild disease variant? From 1999 to 2005, neonatal screening for GCDH deficiency has identified 38 asymptomatic neonates (including 7 low excretors) in Germany and, as far as it is known, no patient has yet been missed. This is in contrast with previous findings from other countries suggesting that some patients with a mild biochemical phenotype may be missed (27). However, this discrepant experience most likely reflects the optimization process while establishing a novel screening method. We have recently demonstrated using data mining strategies that the sensitivity and specificity of MS/MS-based neonatal screening for GCDH deficiency can be increased by implementation of ratios (*i.e.*, C5DC to palmitoylcarnitine or free carnitine) as second tier (28). Thus it is likely to assume that a second tier strategy would improve the identification of low excreting neonates with GCDH deficiency.

It is subject of current debates whether neonatal screening increases the selection of individuals with a mild disease variant who may remain asymptomatic – even if undiagnosed and untreated. Mild disease forms are known for many inborn

errors of metabolism, such as medium-chain acyl-CoA dehydrogenase deficiency (29), isovaleric acidemia (30), or hyperphenylalaninemia (31). However, the natural history of patients with GCDH deficiency does not correlate with the genotype or the biochemical phenotype (32) resulting in a similar *a priori* risk for all untreated patients to suffer acute encephalopathic crises (4,10,11,13). Furthermore, no variables are known that allow predicting the disease course of presymptomatically diagnosed patients and the necessity for treatment. Despite this, we cannot exclude that NBS for GCDH deficiency may identify patients with a mild disease variant. The major source of uncertainty is the discrepancy of the mean prevalences within the historical patients (cohorts I and II) and the NBS group (cohort III). This may be explained by missed patients in the historical cohorts, by selection of patients with a mild disease variant in the NBS cohort or a combination of both. Unfortunately, the number of missed patients in the historical cohort is unknown. Studies on sudden infant death syndrome cannot substitute for this (33) and are likely to underestimate the number of missed cases, since most patients have died after infancy (this study, median age at death: 84 mo).

Since the number of missed patients remains obscure, the ascertainment bias of this study can be estimated by the suggested maximal proportion of patients who may do well without treatment. Assuming that the 1.87-fold higher mean prevalence in cohort III (NBS group) than cohort II (latest historical patients cohort) results from a selection of patients with a mild disease variant by NBS, it can be calculated that 18 patients from the NBS group may have done well without treatment. Even if this was true, absolute risk reduction (0.57 instead of 0.67) and the number needed to treat to prevent one encephalopathic crisis (1.74 instead of 1.50) would still show a beneficial effect of NBS and intensive management. However, it seems doubtful that NBS for GCDH deficiency increases the number of patients who may not require treatment, since 1) presymptomatically diagnosed siblings of symptomatic index patients from the historical patients group show a similar decline of encephalopathic crises as patients from the NBS group, 2) the genotypes and biochemical phenotypes of patients from the NBS group and historical patients group are similar and 3) late-onset type GCDH deficiency has been demonstrated in untreated adolescents or adults (11,12).

In conclusion, the present study demonstrates that NBS in combination with intensive management reduces the risk for the onset of prognostically relevant acute encephalopathic crises in the majority of children with GCDH deficiency and thus confirms recent recommendations for the diagnosis and management of this disease (18). This guideline also describes in detail how maintenance and emergency treatment can be applied. Since no variables are known that reliably predict the clinical course of untreated patients and since treatment efficacy is high in presymptomatically diagnosed patients, there is no known evidence base for a risk stratification and thus it is recommended to treat all patients. Further studies are required to identify the major single aspects of clinical management that influence the outcome of affected patients. However, despite these promising results the long-term outcome of presymptomatically diagnosed children remains to be elucidated.

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REFERENCES

1. Goodman SI, Markey SP, Moe PG, Miles BS, Teng CC 1975 Glutaric aciduria: a "new" disorder of amino acid metabolism. Biochem Med 12:12–21
2. Lindner M, Kölker S, Schulze A, Christensen E, Greenberg CR, Hoffmann GF 2004 Neonatal screening for glutaryl-CoA dehydrogenase deficiency. J Inherit Metab Dis 27:851–859
3. Greenberg CR, Prasad AN, Dilling LA, Thompson JR, Haworth JC, Martin B, Wood-Steinman P, Seargeant LE, Seifert B, Booth FA, Prasad C 2002 Outcome of the first 3-years of a DNA-based neonatal screening program for glutaric acidemia type 1 in Manitoba and northwestern Ontario, Canada. Mol Genet Metab 75:70–78
4. Strauss KA, Puffenberger EG, Robinson DL, Morton DH 2003 Type I glutaric aciduria, part 1: natural history of 77 patients. Am J Med Genet C Semin Med Genet 121:38–52
5. Goodman SI, Stein DE, Schlesinger S, Christensen E, Schwartz M, Greenberg CR, Elpeleg ON 1998 Glutaryl-CoA dehydrogenase mutations in glutaric acidemia (type I): review and report of thirty novel mutations. Hum Mutat 12:141–144
6. Zschocke J, Quak E, Guldberg P, Hoffmann GF 2000 Mutation analysis in glutaric aciduria type I. J Med Genet 37:177–181
7. Christensen E 1983 Improved assay of glutaryl-CoA dehydrogenase in cultured cells and liver: application to glutaric aciduria type I. Clin Chim Acta 129:91–97
8. Hoffmann GF, Athanassopoulos S, Burlina AB, Duran M, de Klerck JB, Lehnert W, Leonard JV, Monavari AA, Müller E, Muntau AC, Naughten ER, Plecko-Starting B, Superti-Furga A, Zschocke J, Christensen E 1996 Clinical course, early diagnosis, treatment, and prevention of disease in glutaryl-CoA dehydrogenase deficiency. Neuropediatrics 27:115–123
9. Bjugstad KB, Goodman SI, Freed CR 2000 Age at symptom onset predicts severity of motor impairment and clinical outcome of glutaric acidemia type 1. J Pediatr 137:681–686
10. Kyllerman M, Skjeldal O, Christensen E, Hagberg G, Holme E, Lonnquist T, Skov L, Rotwelt T, von Dobeln U 2004 Long-term follow-up, neurological outcome and survival rate in 28 Nordic patients with glutaric aciduria type 1. Eur J Paediatr Neurol 8:121–129
11. Kölker S, Garbade SF, Greenberg CR, Leonard JV, Saudubray JM, Ribes A, Kalkanoglu HS, Lund AM, Merinero B, Wajner M, Troncoso M, Williams M, Walter JH, Campistol J, Marti-Herrero M, Caswill M, Burlina AB, Lagler F, Maier EM, Schwahn B, Tokatli A, Dursun A, Coskun T, Chalmers RA, Koeller DM, Zschocke J, Christensen E, Burgard P, Hoffmann GF 2006 Natural history, outcome and therapeutic efficacy in children and adults with glutaryl-CoA dehydrogenase deficiency. Pediatr Res 59:840–847
12. Külkens S, Harting I, Sauer S, Zschocke J, Hoffmann GF, Gruber S, Bodamer OA, Kölker S 2005 Late-onset neurologic disease in glutaryl-CoA dehydrogenase deficiency. Neurology 64:2142–2144
13. Naughten ER, Mayne PD, Monavari AA, Goodman SI, Sulaiman G, Croke DT 2004 Glutaric aciduria type I: outcome in the Republic of Ireland. J Inherit Metab Dis 27:917–920
14. Baric I, Wagner L, Feyh P, Liesert M, Buckel W, Hoffmann GF 1999 Sensitivity and specificity of free and total glutaric acid and 3-hydroxyglutaric measurements by stable-isotope dilution assays for the diagnosis of glutaric aciduria type I. J Inherit Metab Dis 22:867–881
15. Schulze A, Lindner M, Kohlmuller D, Olgemoller K, Mayatepek E, Hoffmann GF 2003 Expanded newborn screening for inborn errors of metabolism by electrospray ionization-tandem mass spectrometry: results, outcome, and implications. Pediatrics 111:1339–1406
16. Chace DH, Kalas TA, Naylor EW 2003 Use of tandem mass spectrometry for multianalyte screening of dried blood spot specimens from newborns. Clin Chem 49:1797–1817

17. Wilcken B, Wiley V, Hammond J, Carpenter K 2003 Screening newborns for inborn errors of metabolism by tandem mass spectrometry. N Engl J Med 348:2304–2312
18. Kölker S, Christensen E, Leonard JV, Greenberg CR, Burlina AB, Burlina AP, Dixon M, Duran M, Goodman SI, Koeller DM, Müller E, Naughten ER, Neumaier-Probst E, Okun JG, Kyllerman M, Surtees RA, Wilcken B, Hoffmann GF, Burgard P 2007 Guideline for the diagnosis and management of glutaryl-CoA dehydrogenase deficiency (glutaric aciduria type I). J Inherit Metab Dis 30:5–22
19. Cole TJ 1990 The LMS method for constructing normalized growth standards. Eur J Clin Nutr 44:45–60
20. Kromeyer-Hauschild K, Wabitsch M, Kunze D, Geller F, Geiβ HC, Hesse V, von Hippel A, Jaeger U, Johnsen D, Korte W, Menner K, Müller D, Müller JM, Niemann-Pilatus A, Remer T, Schaefer F, Wittchen HU, Zabransky S, Zellner K, Ziegler A, Hebebrand J 2001 [Percentiles for the Body measure index for the children and adolescents under consulting different German samples]. Monatsschr Kinderheilkd 149:807–818
21. Prader A, Largo RH, Molinari L, Issler C 1989 Physical growth of Swiss children from birth to 20 years of age. First Zurich longitudinal study of growth and development. Helv Paediatr Acta Suppl 52:1–125
22. Haworth JC, Dilling LA, Seargeant LE 1991 Increased prevalence of hereditary metabolic diseases among native Indians in Manitoba and northwestern Ontario. CMAJ 145:123–129
23. Hoffmann GF, Trefz FK, Barth PG, Böhles JH, Biggemann B, Bremer HJ, Christensen E, Frosch M, Hanefeld F, Hunneman DH, Jacobi H, Kurlemann G, Lawrenz-Wolf B, Rating D, Roe CR, Schutgens RB, Ullrich K, Weisser J, Wendel U, Lehnert W 1991 Glutaryl-coenzyme A dehydrogenase deficiency: a distinct encephalopathy. Pediatrics 88:1194–1203
24. Morton DH, Bennett MJ, Seargeant LE, Nichter CA, Kelley RI 1991 Glutaric aciduria type I: a common cause of episodic encephalopathy and spastic paralysis in the Amish of Lancaster County, Pennsylvania. Am J Med Genet 41:89–95
25. R Development Core Team 2006 R: A language and environment for statistical computing. Vienna: R foundation for statistical computing. URL: http://www.R-project.org, accessed April 18, 2007
26. WHO Multicentre Growth Reference Study Group 2006 WHO Motor Development Study: windows of achievement for six gross motor developmental milestones. Acta Paediatr Suppl 450:86–95
27. Gallagher RC, Cowan TM, Goodman SI, Enns GM 2005 Glutaryl-CoA dehydrogenase deficiency and newborn screening: retrospective analysis of a low excretor provide further evidence that some cases may be missed. Mol Genet Metab 86:417–420
28. Lindner M, Ho S, Fang-Hoffmann J, Hoffmann GF, Kölker S 2006 Neonatal screening for glutaric aciduria type I: Strategies to proceed. J Inherit Metab Dis 29:378–382
29. Andresen BS, Dobrowski SF, O'Reilly L, Muenzer J, McCandless SE, Frazier DM, Udvari S, Bross P, Knudsen I, Banas R, Chace DH, Engel P, Naylor EW, Gregersen N 2001 Medium-chain acyl-CoA dehydrogenase (MCAD) mutations identified by MS/MS-based screening of newborns differ from those observed in patients with clinical symptoms: identification and characterization of a new, prevalent mutation that results in mild MCAD deficiency. Am J Hum Genet 68:1408–1418
30. Ensenauer R, Vockley J, Willard JM, Huey JC, Sass JO, Edland SD, Burton BK, Berry SA, Santer R, Grunert S, Koch HG, Marquardt I, Rinaldo P, Hahn S, Matern D 2004 A common mutation is associated with a mild, potentially asymptomatic phenotype in patients with isovaleric acidemia diagnosed by newborn screening. Am J Hum Genet 75:1136–1142
31. Burgard P, Rupp A, Konecki DS, Trefz FK, Schmidt H, Lichter-Konecki U 1996 Phenylalanine hydroxylase genotypes, predicted residual enzyme activity and phenotypic parameters of diagnosis and treatment of phenylketonuria. Eur J Pediatr 155:S11–S15
32. Christensen E, Ribes A, Merinero B, Zschocke J 2004 Correlation of genotype and phenotype in glutaryl-CoA dehydrogenase deficiency. J Inherit Metab Dis 27:861–868
33. Chace DH, DiPerna JC, Mitchell B, Sgroi B, Hofman LF, Naylor EW 2001 Electrospray tandem mass spectrometry for analysis of acylcarnitines in dried post-mortem blood specimens collected by autopsy from infants with unexplained cause of death. Clin Chem 47:1166–1182

Erratum

In the article, "Maternal Dietary Supplementation with Pomegranate Juice Is Neuroprotective in an Animal Model of Neonatal Hypoxic-Ischemic Brain Injury," by David J. Loren *et al.*, (Pediatr Res 57:858–864) panel A of Fig. 2 was typeset incorrectly. The correct panel, Fig. 2A, appears below. The editors regret the error.