Newborn screening, a disease-changing intervention for glutaric aciduria type 1

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Running Head: Newborn screening for glutaric aciduria type 1

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Abstract

Objective: Untreated individuals with glutaric aciduria type 1 (GA1) commonly present with a complex, predominantly dystonic movement disorder (MD) following acute or insidious onset striatal damage. Implementation of GA1 into newborn screening (NBS) programmes has improved the short-term outcome. It remains unclear, however, whether NBS changes the long-term outcome and which variables are predictive.

Methods: This prospective, observational, multi-centre study includes 87 patients identified by NBS, four patients missed by NBS and three women with GA1 identified by positive NBS results of their unaffected children.

Results: The study population comprises 98.3% of individuals with GA1 identified by NBS in Germany between 1999-2016. Overall, cumulative sensitivity of NBS is 95.6%, but is lower (84%) for patients with low excretor phenotype. Neurologic outcome of patients missed by NBS is as poor as in the pre-NBS era, while the clinical phenotype of diagnosed patients depends on the quality of therapeutic interventions rather than non-interventional variables: Presymptomatic start of treatment according to current guideline recommendations clearly improves the neurologic outcome (MD: 7% of patients), while delayed emergency treatment results in acute onset MD (100%), and deviations from maintenance treatment increase the risk of insidious onset MD (50%). Independent of the neurologic phenotype, kidney function tends to decline with age, a non-neurologic manifestation not predicted by any variable included in this study.

Interpretation: NBS is a beneficial, disease-changing intervention for GA1. However, improved neurologic outcome critically depends on adherence to recommended therapy while kidney dysfunction does not appear to be impacted by recommended therapy.

References to electronic databases

Glutaric aciduria type 1: OMIM # 231670

Glutaryl-CoA dehydrogenase: EC 1.3.8.6

Abbreviations

3OHGA, 3-hydroxyglutaric acid

C5DC, glutarylcarnitine

CI, confidence interval

CKD, chronic kidney disease

EC, encephalopathic crisis

(A)ET, (adequate) emergency treatment (according to guideline recommendations)

GA, glutaric acid

GA1, glutaric aciduria type 1

GFR, glomerular filtration rate

HE, high excretor

HUS, hemolytic uremic syndrome

IQR, interquartile range

LE, low excretor

MD, movement disorder

MS/MS, tandem mass spectrometry

(A)MT, (adequate) maintenance treatment (according to guideline recommendations)

NBS, newborn screening

SDH, subdural hemorrhage

Key words

Glutaric aciduria type 1; glutaric acidemia type 1; newborn screening

Introduction

Glutaric aciduria type 1 (GA1, OMIM #231670) is a rare inherited disorder of L-lysine, L-hydroxylysine and L-tryptophan metabolism due to deficiency of glutaryl-CoA dehydrogenase (EC 1.3.8.6) resulting in accumulation of glutaryl-CoA and its dicarboxylic derivatives, glutaric acid (GA), 3-hydroxyglutaric acid (3OHGA), glutaconic acid and glutarylcarnitine (C5DC) in body tissues, especially within the brain. The estimated incidence in Germany is 1 in 112,700 newborns [95% confidence interval (CI), 1 in 129,455-95,953].[1] Between ages 3-36 months, most untreated patients develop a complex movement disorder (MD) with predominant dystonia due to bilateral striatal damage associated with high morbidity and mortality.[2, 3] This prognostically relevant event may occur *acutely* following an acute encephalopathic crisis (EC)[3] or *insidiously* without preceding crisis.[1, 4, 5] Due to the primarily neurologic phenotype, GA1 is considered a *cerebral* organic aciduria. However, peripheral nervous system[6] and kidneys[7] might also be involved in the long-term disease course. Two arbitrarily defined biochemical subgroups, *low* (LE) and *high excretors* (HE)[8], have been described according to the amount of urinary GA, both sharing the same risk of developing MD if untreated.[3, 9] However, studies revealed a higher frequency of progressive extrastriatal CNS abnormalities in HE patients, though their clinical relevance is unclear.[10, 11]

Metabolic treatment consisting of a low lysine diet and carnitine supplementation for maintenance treatment (MT) and intermittent emergency treatment (ET) during episodes likely to induce catabolism such as infections, has improved neurologic short-term outcome in early diagnosed individuals in most studies.[1, 3, 12-16] Evidence-based recommendations have been developed and recently revised.[17, 18] Noteworthy, treatment reduces cerebral accumulation of putatively neurotoxic dicarboxylic metabolites and improves outcome in Gcdh-deficient mice thereby linking neurotoxicity to the clinical phenotype.[19, 20]

Since C5DC can be detected by electrospray-ionisation tandem mass spectrometry (MS/MS) and early treatment is thought to be effective, GA1 has been increasingly included in national newborn screening (NBS) programmes.[21, 22] In Germany, pilot NBS projects for GA1 were initiated in single metabolic centres in 1999, followed by nationwide start of a MS/MS-based NBS in 2005. The prospective observational study on GA1 patients identified by NBS started in 1999 and, to our knowledge, follows the largest cohort of early diagnosed and treated patients worldwide. Previous interim analyses of this longitudinal study have improved our knowledge about this rare disease demonstrating that NBS and adherence to guideline recommendations improves the short-term outcome of affected individuals.[1, 16] However, impact on long-term outcome is unknown. The major aims of this study are to investigate long-term neurologic and non-neurologic outcome in patients treated and prospectively followed after positive NBS, to identify major disease-modifying effects of interventional and non-interventional parameters and to evaluate the overall benefit of the NBS programme for GA1 patients.

Methods

Study population

The national prospective, multi-centre, non-randomised, non-controlled observational study includes patients diagnosed with GA1 between January 1st, 1999 and July 1st, 2016 (**Suppl. Tab. 1, 2**). Inclusion criteria comprised (1) patients identified by NBS, or patients missed by NBS and later diagnosed due to symptoms or symptomatic siblings, or undiagnosed women identified by positive NBS result of their unaffected child (maternal GA1), and (2) confirmation of diagnosis by quantitative analysis of urinary 3OHGA and/or *GCDH* gene analysis and/or quantitative analysis of residual activity of glutaryl-CoA dehydrogenase, and (3) written informed consent from patients and/or parents. Individuals not fulfilling these criteria were excluded.

Biochemical phenotype (HE or LE) was classified according to a previous definition.[8] Urinary GA is above 100 mmol/mol creatinine in HE patients, which is associated with residual enzyme activity of 0-2%. Urinary GA is below 100 mmol/mol creatinine or even normal in LE patients, who have residual enzyme activity of 3-30%.[9] Hydroxyglutaric acid is usually elevated in both subtypes. Confirmatory diagnostics comprised quantification of urinary concentrations of GA and 3OHGA, molecular genetic analysis of *GCDH* gene and/or analysis of residual enzyme activity in leucoytes (Dr Wibrand, Metabolic Laboratory, Department of Clinical Genetics, Copenhagen, Denmark). "Migration" was defined by at least one parent being born in another country than Germany.

The study was approved by the Institutional Ethics Committee of the coordinating centre (University Hospital Heidelberg, application no. S-525/2010) and all contributing study sites. For four patients who were lost to follow-up after the previous interim analysis in 2009[1] data of their last visit were used. Previous interim analyses of this study were also approved by the Institutional Ethics Committee (application no. 314/2002 and S-525/2010). All patients and/or parents have given written informed consent.

Treatment

MT according to guideline recommendations[18] consists of (1) neonatal start of age-adapted low lysine diet with supplementation of a lysine-free, tryptophan-reduced, arginine-containing amino acid supplement for all patients up to age 6 years, (2) protein-controlled nutrition for all patients above 6 years using natural protein with a low lysine content and avoiding lysine-rich food, and (3) lifelong oral carnitine supplementation. ET according to guideline recommendations consists of carbohydrate-enriched, low to no-protein protocol intermittently used during potentially catabolic episodes and initiated within 24 hours after onset of alarming symptoms (e.g. fever, vomiting, feeding problems). Treatments were classified as *adequate* (AMT, AET), only if they complied with guideline recommendations throughout the patient's course. Patients were classified as being *supervised by a metabolic centre* if supervision had started during the neonatal period and continued until the last documented follow-up visit.

Outcome variables

Clinical and biochemical follow-up parameters were assessed prospectively. Neurologic manifestations were separated into two categories: (1) major motor symptoms, i.e. manifestation of a MD, and (2) minor motor symptoms, i.e. fine motor deficits and/or delayed achievement of motor milestones in the absence of MD.

Asymptomatic individuals had neither major nor minor symptoms. MD was classified as *mild* if children showed no significant disability in daily life despite some degree of motor dysfunction and gross motor milestones were achieved in time or with slight delay), *moderate* if motor dysfunction caused disability but despite this some motor functions were preserved and *severe* if the MD caused important disability with few motor skills left with gross motor milestones not or only partially achieved.[1] Onset type of MD was classified as (1) *acute onset with encephalopathic crisis (EC),* (2) *acute onset with subdural hemorrhage* (SDH), or (3) *insidious onset* without an apparent acute event. Acute EC was defined as acute onset of a complex, predominantly dystonic MD after an episode that is likely to precipitate catabolism (e.g., febrile illness) during infancy or childhood in the absence of known alternative causes (e.g., meningitis). Renal outcome was assessed by estimated glomerular filtration rate (GFR) according to Schwartz.[23]

Statistical analysis

Independent variables used for outcome analysis comprised (1) adherence to MT, (2) adherence to ET, (3) gender, (4) biochemical subtype, (5) migration, and (6) supervision by a metabolic centre.

Analyses were computed with the statistical package R.[24] Survival was estimated using the Kaplan-Meier method. If a patient was reported to be alive at last follow-up visit, patient data were censored. The log-rank test was applied to compare potential differences between analysed subgroups. Unbiased recursive partitioning was used to determine the impact of independent variables on outcome variables.[25] Count data was analysed with log-linear models and visualised with Pearson residual shaded mosaic graphs.[26] Age of onset of symptoms between two groups was compared with Mann-Whitney Test.

Role of the funding source

Third-party donors of the study were not involved in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Study sample and epidemiology

The study sample includes 94 individuals with confirmed diagnosis of GA1, of whom 87 (42 male, 45 female) were identified by NBS, four female patients missed by NBS and three women with undiagnosed GA1 identified by positive NBS results of their unaffected children (maternal GA1) (**Suppl. Tables 1 and 2**). The four patients missed by NBS all had LE phenotype and normal NBS results. Three of them were later on identified after acute onset of severe (n=2) or moderate (n=1) dystonia following an EC. One patient with severe dystonia died at age 3 years, while her twin sibling was diagnosed at age 390 days, still being asymptomatic. It remains unknown whether additional patients were missed by NBS during the study interval. Unexpectedly, positive NBS results of unaffected newborns helped to identify three women with undiagnosed GA1. Two of them were asymptomatic at diagnosis (except chronic headaches) while one presented with coordination deficits and mild cognitive disability. All late diagnosed women had HE phenotype. Pregnancies and deliveries were uneventful. Their children were asymptomatic and showed normal development virtually excluding significant metabolic embryofetopathy.

In the NBS group, median age at diagnosis was 7 days [range 2-217, interquartile range (IQR) 6-10 days]. Median age at last visit was 8.2 years (range 0.73-17·75, IQR 4.37-12.45 years) and cumulative follow-up time of all patients was 710.6 years. Seventy-one of 87 NBS patients (82%) were older than three years at last visit. All NBS patients were asymptomatic at diagnosis except one (ID 75) with neonatal infection but without developing irreversible neurologic symptoms. Overall, sixty-four patients (73.5%) had HE and 21 patients (24%) LE phenotype. In two patients, biochemical phenotype was not reported. Positive NBS results were confirmed by *GCDH* gene analysis and/or enzyme activity analysis in 80, whereas in seven patients diagnosis was exclusively confirmed by quantitative analysis of urinary 3OHGA. Thirty-eight patients (44%) were of German origin, while 49 patients (56%) had migrational background, mostly from Turkey (n=21).

The German National Society for Newborn Screening reported on 61 confirmed patients from 2004-2015 (no data were available for 1999-2003), with 60 of them (98.3%) being included in this study, confirming its high representativeness. Based on correctly identified and missed patients between 1999-2016, estimated incidence of GA1 in Germany is 1 in 124,930 newborns (95% CI: 124,845-125,015) and overall sensitivity of NBS for GA1 was 95.6% but with discrepancy between patients with HE (100%) and LE (84%) phenotype.

Genotype phenotype correlation

Molecular genetic results were available for 76 patients of the NBS group, for 53 patients both nucleotide and protein changes and for 23 patients only protein changes of gene variations were reported. In three patients, two with HE and one with LE phenotype, only a single gene variation was detected, while one patient (HE) had three variants. Thirty-five patients were homozygous and 38 compound heterozygous. The majority of homozygous patients were HE (32/35). Among them, p.Glu365Lys (n=9 patients; ExAC allele frequency: 0.00001652), p.Arg402Trp (n=5; allele frequency 0.0002233), p.Ala421Val (n=2; allele frequency: 0.0001896) and p.Pro248Leu (n=2; not listed in ExAc), known to cause HE, were most frequently found. Compound heterozygous patients were HE (n=20) or LE (n=16), depending on the cumulative residual activity of the two

alleles (**Suppl. Table 2**). Although the genotype clearly predicted the biochemical phenotype, neither genotype nor biochemical phenotype predicted the clinical phenotype. For instance, the frequency of dystonia did not differ in patients with HE (31%) and LE (19%; p=0.3048) confirming previous studies.[1, 9, 16] In line with previous studies,[1, 12, 15, 16] we did not find evidence that NBS for GA1 systematically identifies individuals with a clinically benign phenotype not requiring treatment such as in isovaleric aciduria[27] and hyperphenylalaninemia.[28]

Metabolic therapy and long-term management

Seventy-four NBS patients (85%) were followed by one of 17 contributing metabolic centres. In addition, five patients were followed by local hospitals and eight patients had sporadic follow-ups. Fifty-nine patients (68%) were treated in full accordance to previously published recommendations for MT and ET[17, 18] while 28 patients (32%) were not. Deviations in MT (n=16) comprised of non-adherence to the diet (n=8), inadequate dietary prescription (n=6), feeding problems (n=1), and delayed start of MT (n=1). ET was delayed for more than 24 hours in 12 patients during at least one episode of febrile illness. All patients received oral carnitine supplementation up to age 6 years.

Neurologic outcome is predicted by interventional variables

Fifty-six patients of the NBS group (64%) remained asymptomatic while 31 patients (36%) developed neurologic symptoms: 26 of them (30%) had major motor symptoms (MD) and five (6%) minor motor symptoms.

Major motor symptoms

Among 26 patients with major motor symptoms, 13 developed acute onset MD following EC (n=11) precipitated by febrile illness at a median age of 270 days (range 147-570 days) or SDH (n=2) following minor head trauma. Another 12 patients developed insidious onset MD at a median age of 630 days (range 180-1680 days) without preceding acute events, and in one patient onset type was unclear. Age at onset differed in patients with acute and insidious onset (p=0.012). All patients with MD developed dystonia (with additional chorea in four patients), except two patients with spasticity following acute SDH and one patient with ataxia. Dystonia was more severe after acute onset with EC (severe: 6, moderate: 4, mild: 1 patient) than after insidious onset (severe: 1, moderate: 3, mild: 8 patients). In analogy, severity of dystonia decreased with age at onset and did manifest more frequently before than after age 3 years (p<0.001).

Among the 59 patients showing full adherence to recommendations for MT and ET, 55 (93%) remained asymptomatic and four developed moderate (n=2) or mild (n=2) dystonia. In contrast, all patients (n=12) without adherence to ET developed severe (n=6), moderate (n=4) or mild (n=2) dystonia, mostly with acute onset (10/12 patients), demonstrating the strong effect of ET on dystonia (p<0.001) (**Fig. 1**). In analogy to ET but less pronounced, deviations from MT (n=16 patients) also increased the frequency of dystonia (p=0.0003, **Fig. 1**). Eight of them (50%) developed insidious onset dystonia which was mild (n=6) or moderate (n=2).

Since the achievement of head control is an early motor milestone, we tested whether head lag might serve as an early predictor of MD in GA1. In the NBS group, the majority of individuals (n=70) achieved normal head control, while eight individuals achieved this motor milestone but lost it again and three individuals never achieved it. Eight of the 11 patients with abnormal head control lost it following acute onset dystonia, while

most patients with insidious onset dystonia (n=8) had normal head control. Eight children with abnormal head control did not adhere to ET, confirming the impact of therapy on motor outcome.

Minor motor symptoms

Five patients without MD developed minor motor symptoms at a median age of 330 days (range 180-2950 days) comprising motor developmental delay (n=2), fine motor and coordination deficits (n=2) or slowed motor functions (n=1). Four of them did not receive MT according to recommendations before age 6 years showing that non-adherence to MT increased the risk of minor motor symptoms (**Fig. 2**).

Epilepsy

One patient with severe brain damage following acute SDH presented with bilateral tonic-clonic seizures at age 4 years which were pharmacologically controlled by oxcarbazepine. Another patient missed by NBS and diagnosed at age 390 days while being asymptomatic, developed benign epilepsy with centro-temporal spikes (Rolandic epilepsy) but did not require antiepileptic treatment.

Phenotypic extension

Recently, peripheral polyneuropathy and chronic kidney disease (CKD) were reported for adult patients with GA1[6, 7] extending the phenotypic spectrum. In this study, however, polyneuropathy was not detected. One patient published previously[29] presented with fatal acute renal failure due to hemolytic uremic syndrome (HUS) precipitated by pneumococcal infection. Regardless of the neurologic phenotype, overall GFR [median at last visit: 123 ml/min per 1.73 m^2 (85-214)] declined with age (**Fig. 3**). At last visit, three of the six patients older than 12 years had a GFR below 90 ml/min per 1.73 m^2 (CKD stage 2). Eight patients showed intermittently reduced GFR below 90 and two patients below 60 ml/min per 1.73 m^2 (CKD stage 3a). GFR did not differ between LE and HE patients (**Fig. 3**) or treatment groups (not shown).

Survival

Eighty-two NBS patients (93%) survived (**Fig. 4**). Of the five patients (7%) who deceased [median (range) age: 3.32 (0.87-6.07) years] four had dystonia (severe: 3, moderate: 1) following EC. Two of them died during severe infectious diseases, one with bacterial sepsis and meningitis and another with influenza type B infection, while in the other two patients the cause of death remained unknown. The fifth patient[29], hitherto asymptomatic, died during HUS precipitated by pneumococcal infection. Overall, patients with severe MD had a lower life expectancy than all other patients ($p<0.001$; **Fig. 4**). In analogy, the survival rate was lower in patients with acute onset MD compared to those with insidious onset and no MD ($p<0.0001$). Deviations from ET recommendations had a major negative impact on survival ($p=0.0036$) while gender, biochemical subtype, migration, and follow-up by metabolic centre had no measurable effects.

Discussion

The main findings of this long-term multi-centre observational study including 87 patients with confirmed diagnosis of GA1, i.e. 98.3% of patients identified by NBS in Germany between 1999-2016, four patients missed by NBS and three women with maternal GA1, are: (1) NBS and neonatal start of and adherence to recommended treatment results in better neurologic outcome than late diagnosis and non-adherence. (2) Neurologic, but not non-neurologic outcome depends on presymptomatic diagnosis (NBS) and therapeutic interventions (MT, ET) rather than non-interventional variables (genotype, biochemical phenotype, gender, migration). However, minor accidental head trauma causing acute SDH is an alternative cause of neurologic disease despite adherence to recommended therapy. (3) C5DC screening has an estimated cumulative sensitivity of 95.6%, but is lower (84%) for patients with LE phenotype. Patients missed by NBS are confronted with high morbidity and mortality, similar to the pre-NBS era. (4) Natural history of GA1 is still incompletely understood. Decline of GFR highlights increased risk of developing CKD in children and adolescents. Early diagnosis and treatment do not seem to prevent renal manifestation.

NBS for GA1: the prerequisite to improve neurologic outcome and survival

Since previous studies have shown that neonatal diagnosis and start of treatment improve neurologic short-term outcome and survival of GA1 patients,[1, 3, 12, 14, 30] GA1 has been increasingly included in national NBS programmes worldwide.[21, 22] We hereby demonstrate the beneficial long-term effect of NBS for GA1 in the largest NBS cohort covering more than 98.3% of patients identified by NBS in Germany and longest follow-up reported so far (cumulative follow-up time: 711 years). Notwithstanding this great achievement, patients with LE might still be missed and have a poor outcome[1, 31, 32] proving that, unlike in other inherited metabolic diseases,[27, 28] LE phenotype should not be misinterpreted as a benign disease variant. Untreated patients with HE and LE both share the same high *a priori* risk of striatal damage[3] and, therefore, therapeutic recommendations for all patients identified by NBS are identical.[18] Recent observations have revealed a higher frequency of extrastriatal abnormalities, progressive neuroaxonal compromise and increased cerebral GA concentrations in HE patients progressing with age but clinical relevance is unclear.[10, 11] Of note, we did not detect differences in the clinical course between HE and LE patients either. To improve the sensitivity of NBS for LE patients, a genetic NBS programme was established for the First Nations in Ontario and Manitoba, a known high-risk GA1 population with LE phenotype.[33] However, since this approach would not be suitable for populations with multiple, mostly private gene variations, sensitivity and specificity of MS/MS-based NBS has meanwhile been improved by introduction of multiple reaction monitoring, adjustment of cut-offs,[34] and additional use of C5DC-to-octanoyl and C5DC-to-palmitoylcarnitine ratios. Nevertheless, more efforts are still required to reliably identify LE patients.[35-37]

Although not intended, NBS helped identifying women with undiagnosed GA1 (maternal GA1)[11, 38-40] following workup of initially abnormal NBS in their unaffected children as recommended by the current guideline,[18] unravelling the close coupling of maternal and fetal metabolism. Since affected mothers may be asymptomatic or show unspecific symptoms like headaches maternal GA1 might be underdiagnosed. All identified mothers, as well as all reported late diagnosed GA1 patients so far[11], had HE phenotype. Since HE patients are at risk for progredient extrastriatal abnormalities such as signal changes of supratentorial white matter and

subependymal nodules[10, 11], identification and start of treatment in these patients aiming at reducing accumulation of neurotoxic metabolites is important.

Reported pregnancies and deliveries of women with GA1 were unremarkable confirming previous case reports.[38, 39] At present, there is no evidence for metabolic decompensation of untreated women with GA1 during pregnancy or childbirth and following delivery and for metabolically induced embryofetopathy such as in maternal phenylketonuria.[41]

Phenotypic diversity mostly relies on therapeutic variations

Phenotypic diversity is found in all diseases reflecting genetic, adaptive and environmental influences. For untreated GA1 patients, the severity of the clinical phenotype cannot be reliably predicted based on the genotype or biochemical phenotype.[3, 9] Even siblings with the same *GCDH* gene variant may have discrepant disease courses,[1, 16] and a few untreated individuals remain asymptomatic until adulthood.[11, 42] This points to a multifactorial, not yet fully understood mechanism being in line with studies in Gcdh-deficient mice.[43] However, up to 90-95% of untreated individuals develop striatal damage[44] indicating a high penetrance of pathogenic *GCDH* gene variants. Therapeutic interventions do not significantly influence outcome of symptomatically diagnosed patients, since striatal damage is irreversible.[45]

For patients diagnosed by NBS while being asymptomatic, specific therapeutic modifications have significantly changed the disease course. Metabolic treatment, specifically adherence to MT and ET according to recommendations,[18, 46] best predicts outcome. In this study, 93% of patients identified by NBS and receiving metabolic treatment according to guideline recommendations remained asymptomatic. This is in line with previous reports from different countries, all focusing on short-term outcome. [1, 3, 12, 14, 15] Although a national guideline is available in Germany (www.awmf.org; register no. 027-018) and studies have confirmed the short-term benefit of metabolic treatment, an unexpectedly high frequency of treatment deviations (32% of patients) was detected. Neurologic disease was most pronounced in patients receiving inadequate ET resulting in acute onset dystonia in almost all cases, primarily affecting children below age three years and accounting for 13% of all patients identified by NBS.[1, 16] Inadequate ET also had a negative impact on survival. Delayed start of ET during potentially threatening episodes of febrile illness was the major management problem and highlights the need for improved communication with and education of parents but also extended proclamation of guideline recommendations.

Another 12 patients (13%) developed insidious onset dystonia, a disease course which has been increasingly described after implementation of NBS programmes,[1, 3, 4] usually appearing later and being clinically less severe than *acute* onset dystonia. It had been postulated that perinatal events might induce the manifestation of insidous onset dystonia[47], however, the earliest manifestation of MD in the 12 insidious onset patients in our cohort was 180 days with a median age at onset of 630 days making causal perinatal events less likely in these patients. We identified inadequate MT as the main risk factor for insidious onset dystonia, with non-adherence of parents to prescribed MT and non-compliance of physicians with existing guidelines being most frequent deviations. Impact of MT on outcome is supported by another study demonstrating significantly improved outcome after revision of dietary management.[14] Inadequate MT was also the major risk factor of developing

minor motor symptoms suggesting a continuous phenotypic spectrum precipitated by inadequate MT, in particular inadequate diet.

Extending the clinical phenotype to non-neurological disease manifestation

CKD has recently been reported as the first non-neurologic disease manifestation in adult GA1 patients.[7] In addition, single patients presented with acute renal failure[48] and nephrotic syndrome[49]. Renal manifestation was also demonstrated in Gcdh-deficient mice.[50] Interference of GA and 3OHGA with transport of organic anions and dicarboxylic acids in proximal tubular epithelial cells was postulated as underlying mechanism.[51] We observed a slow decline, starting in childhood to adolescence. Three patients had CKD stage 2, another ten patients had intermittent GFR abnormalities (CKD stage 2 or 3a, no patient underwent dialysis), highlighting the necessity of regular monitoring of renal function in this age group.[18] Noteworthy, CKD did not depend on the biochemical phenotype and the mode of therapy. Since kidney dysfunction was found in patients with or without recommended therapy, a causal link between kidney dysfunction and metabolic therapy seems unlikely, but cannot be excluded by this study.

Conclusion

This study on the largest prospectively followed cohort of early diagnosed and treated GA1 patients worldwide demonstrates that the beneficial effect of NBS programmes for GA1 critically depends on the diagnostic quality and adherence to recommended metabolic therapy. Deviations at any step of this complex process may hamper the long-term benefit resulting in high morbidity and mortality, similar to the pre-NBS era. Since a significant number of early diagnosed patients developed irreversible neurologic symptoms as a consequence of therapeutic non-adherence and since with increasing age CKD may manifest despite adherence to therapy, this study also highlights the need for optimised therapies. Finally, it demonstrates that long-term observational studies are a valid tool to evaluate NBS programmes for rare diseases.

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Author contributions

N.B., K.M., and S.K. contributed to the conception and design of the study. N.B., K.M., E.T., K.A.S., T.M., N.W., I.M., A.M.D., P.F., S.C.G., J.V., R.S., M.R.B., S.B., A.D., A.N., M.L., J.H., G.F.H., C.M., E.M.M., R.E., S.F.G., and S.K. contributed to the acquisition and analysis of data. N.B., S.F.G., and S.K. contributed to drafting the text and preparing the figures.

Potential conflicts of interests

Nothing to report.

References

1. Heringer J, Boy SP, Ensenauer R, et al. Use of guidelines improves the neurological outcome in glutaric aciduria type I. Annals of neurology. 2010 Nov;68(5):743-52.

2. Kyllerman M, Skjeldal O, Christensen E, et al. Long-term follow-up, neurological outcome and survival rate in 28 Nordic patients with glutaric aciduria type 1. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society. 2004;8(3):121-9.

3. Kolker S, Garbade SF, Greenberg CR, et al. Natural history, outcome, and treatment efficacy in children and adults with glutaryl-CoA dehydrogenase deficiency. Pediatric research. 2006 Jun;59(6):840-7.

4. Busquets C, Merinero B, Christensen E, et al. Glutaryl-CoA dehydrogenase deficiency in Spain: evidence of two groups of patients, genetically, and biochemically distinct. Pediatric research. 2000 Sep;48(3):315-22.

5. Hoffmann GF, Athanassopoulos S, Burlina AB, et al. Clinical course, early diagnosis, treatment, and prevention of disease in glutaryl-CoA dehydrogenase deficiency. Neuropediatrics. 1996 Jun;27(3):115-23.

6. Herskovitz M, Goldsher D, Sela BA, Mandel H. Subependymal mass lesions and peripheral polyneuropathy in adult-onset glutaric aciduria type I. Neurology. 2013 Aug 27;81(9):849-50.

7. Kolker S, Valayannopoulos V, Burlina AB, et al. The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 2: the evolving clinical phenotype. Journal of inherited metabolic disease. 2015 Nov;38(6):1059-74.

8. Baric I, Wagner L, Feyh P, Liesert M, Buckel W, Hoffmann GF. Sensitivity and specificity of free and total glutaric acid and 3-hydroxyglutaric acid measurements by stable-isotope dilution assays for the diagnosis of glutaric aciduria type I. Journal of inherited metabolic disease. 1999 Dec;22(8):867-81.

9. Christensen E, Ribes A, Merinero B, Zschocke J. Correlation of genotype and phenotype in glutaryl-CoA dehydrogenase deficiency. Journal of inherited metabolic disease. 2004;27(6):861-8.

10. Harting I, Boy N, Heringer J, et al. (1)H-MRS in glutaric aciduria type 1: impact of biochemical phenotype and age on the cerebral accumulation of neurotoxic metabolites. Journal of inherited metabolic disease. 2015 Sep;38(5):829-38.

11. Boy N, Heringer J, Brackmann R, et al. Extrastriatal changes in patients with late-onset glutaric aciduria type I highlight the risk of long-term neurotoxicity. Orphanet journal of rare diseases. 2017 Apr 24;12(1):77.

12. Couce ML, Lopez-Suarez O, Boveda MD, et al. Glutaric aciduria type I: outcome of patients with early- versus late-diagnosis. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society. 2013 Jul;17(4):383-9.

13. Monavari AA, Naughten ER. Prevention of cerebral palsy in glutaric aciduria type 1 by dietary management. Archives of disease in childhood. 2000 Jan;82(1):67-70.

14. Strauss KA, Brumbaugh J, Duffy A, et al. Safety, efficacy and physiological actions of a lysine-free, arginine-rich formula to treat glutaryl-CoA dehydrogenase deficiency: focus on cerebral amino acid influx. Molecular genetics and metabolism. 2011 Sep-Oct;104(1-2):93-106.

15. Viau K, Ernst SL, Vanzo RJ, Botto LD, Pasquali M, Longo N. Glutaric acidemia type 1: outcomes before and after expanded newborn screening. Molecular genetics and metabolism. 2012 Aug;106(4):430-8.

16. Kolker S, Garbade SF, Boy N, et al. Decline of acute encephalopathic crises in children with glutaryl-CoA dehydrogenase deficiency identified by newborn screening in Germany. Pediatric research. 2007 Sep;62(3):357-63.

17. Kolker S, Christensen E, Leonard JV, et al. Diagnosis and management of glutaric aciduria type I--revised recommendations. Journal of inherited metabolic disease. 2011 Jun;34(3):677-94.

18. Boy N, Muhlhausen C, Maier EM, et al. Proposed recommendations for diagnosing and managing individuals with glutaric aciduria type I: second revision. Journal of inherited metabolic disease. 2017 Jan;40(1):75-101.

19. Sauer SW, Opp S, Hoffmann GF, Koeller DM, Okun JG, Kolker S. Therapeutic modulation of cerebral L-lysine metabolism in a mouse model for glutaric aciduria type I. Brain : a journal of neurology. 2011 Jan;134(Pt 1):157-70.

20. Zinnanti WJ, Lazovic J, Housman C, et al. Mechanism of age-dependent susceptibility and novel treatment strategy in glutaric acidemia type I. The Journal of clinical investigation. 2007 Nov;117(11):3258-70.

21. Loeber JG, Burgard P, Cornel MC, et al. Newborn screening programmes in Europe; arguments and efforts regarding harmonization. Part 1. From blood spot to screening result. Journal of inherited metabolic disease. 2012 Jul;35(4):603-11.

22. Horster F, Kolker S, Loeber JG, Cornel MC, Hoffmann GF, Burgard P. Newborn Screening Programmes in Europe, Arguments and Efforts Regarding Harmonisation: Focus on Organic Acidurias. JIMD reports. 2017;32:105-15.

23. Schwartz GJ, Munoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. Journal of the American Society of Nephrology : JASN. 2009 Mar;20(3):629-37.

24. RTeam. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2017.

25. Hothorn T. HK, Zeileis A. Unbiased Recursive Partitioning: A Conditional Inference Framework. J Comput Graph Stat. 2006;15(3):651-74.

26. Meyer D ZA, Hornik K. vcd: Visualizing Categorical Data. R package version 1.4-3. 2016.

27. Ensenauer R, Vockley J, Willard JM, et al. A common mutation is associated with a mild, potentially asymptomatic phenotype in patients with isovaleric acidemia diagnosed by newborn screening. American journal of human genetics. 2004 Dec;75(6):1136-42.

28. Weglage J, Pietsch M, Feldmann R, et al. Normal clinical outcome in untreated subjects with mild hyperphenylalaninemia. Pediatric research. 2001 Apr;49(4):532-6.

29. du Moulin M, Thies B, Blohm M, et al. Glutaric Aciduria Type 1 and Acute Renal Failure: Case Report and Suggested Pathomechanisms. JIMD reports. 2017 Jul 12.

30. Bijarnia S, Wiley V, Carpenter K, Christodoulou J, Ellaway CJ, Wilcken B. Glutaric aciduria type I: outcome following detection by newborn screening. Journal of inherited metabolic disease. 2008 Aug;31(4):503-7.

31. Gallagher RC, Cowan TM, Goodman SI, Enns GM. Glutaryl-CoA dehydrogenase deficiency and newborn screening: retrospective analysis of a low excretor provides further evidence that some cases may be missed. Molecular genetics and metabolism. 2005 Nov;86(3):417-20.

32. Treacy EP, Lee-Chong A, Roche G, Lynch B, Ryan S, Goodman S. Profound neurological presentation resulting from homozygosity for a mild glutaryl-CoA dehydrogenase mutation with a minimal biochemical phenotype. Journal of inherited metabolic disease. 2003;26(1):72-4.

33. Greenberg CR, Prasad AN, Dilling LA, et al. Outcome of the first 3-years of a DNA-based neonatal screening program for glutaric acidemia type 1 in Manitoba and northwestern Ontario, Canada. Molecular genetics and metabolism. 2002 Jan;75(1):70-8.

34. McHugh D, Cameron CA, Abdenur JE, et al. Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: a worldwide collaborative project. Genetics in medicine : official journal of the American College of Medical Genetics. 2011 Mar;13(3):230-54.

35. Lindner M, Ho S, Fang-Hoffmann J, Hoffmann GF, Kolker S. Neonatal screening for glutaric aciduria type I: strategies to proceed. Journal of inherited metabolic disease. 2006 Apr-Jun;29(2-3):378-82.

36. Estrella J, Wilcken B, Carpenter K, Bhattacharya K, Tchan M, Wiley V. Expanded newborn screening in New South Wales: missed cases. Journal of inherited metabolic disease. 2014 Nov;37(6):881-7.

37. Moore T, Le A, Cowan TM. An improved LC-MS/MS method for the detection of classic and low excretor glutaric acidemia type 1. Journal of inherited metabolic disease. 2012 May;35(3):431-5.

38. Crombez EA, Cederbaum SD, Spector E, et al. Maternal glutaric acidemia, type I identified by newborn screening. Molecular genetics and metabolism. 2008 May;94(1):132-4.

39. Garcia P, Martins E, Diogo L, et al. Outcome of three cases of untreated maternal glutaric aciduria type I. European journal of pediatrics. 2008 May;167(5):569-73.

40. Vilarinho L, Rocha H, Sousa C, et al. Four years of expanded newborn screening in Portugal with tandem mass spectrometry. Journal of inherited metabolic disease. 2010 Dec;33 Suppl 3:S133-8.

41. Lenke RR, Levy HL. Maternal phenylketonuria and hyperphenylalaninemia. An international survey of the outcome of untreated and treated pregnancies. The New England journal of medicine. 1980 Nov 20;303(21):1202-8.

42. Bahr O, Mader I, Zschocke J, Dichgans J, Schulz JB. Adult onset glutaric aciduria type I presenting with a leukoencephalopathy. Neurology. 2002 Dec 10;59(11):1802-4.

43. Sauer SW, Opp S, Komatsuzaki S, et al. Multifactorial modulation of susceptibility to l-lysine in an animal model of glutaric aciduria type I. Biochimica et biophysica acta. 2015 May;1852(5):768-77.

44. Strauss KA, Puffenberger EG, Robinson DL, Morton DH. Type I glutaric aciduria, part 1: natural history of 77 patients. American journal of medical genetics Part C, Seminars in medical genetics. 2003 Aug 15;121C(1):38-52.

45. Bjugstad KB, Goodman SI, Freed CR. Age at symptom onset predicts severity of motor impairment and clinical outcome of glutaric acidemia type 1. The Journal of pediatrics. 2000 Nov;137(5):681-6.

46. Kolker S, Christensen E, Leonard JV, et al. Guideline for the diagnosis and management of glutaryl-CoA dehydrogenase deficiency (glutaric aciduria type I). Journal of inherited metabolic disease. 2007 Feb;30(1):5-22.

47. Strauss KA, Lazovic J, Wintermark M, Morton DH. Multimodal imaging of striatal degeneration in Amish patients with glutaryl-CoA dehydrogenase deficiency. Brain : a journal of neurology. 2007 Jul;130(Pt 7):1905-20.

48. Pode-Shakked B, Marek-Yagel D, Rubinshtein M, et al. Glutaric Aciduria type I and acute renal failure - Coincidence or causality? Molecular genetics and metabolism reports. 2014;1:170-5.

49. Poge AP, Autschbach F, Korall H, Trefz FK, Mayatepek E. Early clinical manifestation of glutaric aciduria type I and nephrotic syndrome during the first months of life. Acta paediatrica. 1997 Oct;86(10):1144-7.

50. Thies B, Meyer-Schwesinger C, Lamp J, et al. Acute renal proximal tubule alterations during induced metabolic crises in a mouse model of glutaric aciduria type 1. Biochimica et biophysica acta. 2013 Oct;1832(10):1463-72.

51. Hagos Y, Krick W, Braulke T, Muhlhausen C, Burckhardt G, Burckhardt BC. Organic anion transporters OAT1 and OAT4 mediate the high affinity transport of glutarate derivatives accumulating in patients with glutaric acidurias. Pflugers Archiv : European journal of physiology. 2008 Oct;457(1):223-31.

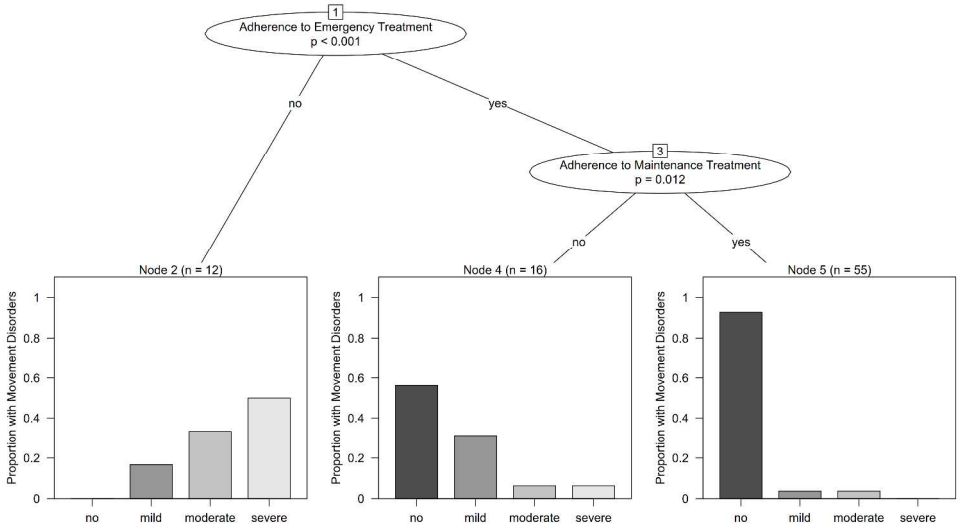
Figure Legends

Fig 1. Effect of treatment adherence on development of movement disorder using recursive partitioning. Non-adherence to recommendations for emergency treatment has the strongest impact on outcome since patients usually develop a severe movement disorder (node 2), independently from adherence to maintenance treatment. Patients not following recommendations for maintenance treatment have an increased risk for mostly mild insidious onset dystonia (node 4). The majority of patients who adhere to emergency and maintenance treatment remain asymptomatic (node 5).

Fig 2. Effect of treatment adherence on development of major motor (movement disorder) and minor motor symptoms using recursive partitioning. Patients without adherence to recommendations for emergency treatment all develop major motor symptoms (node 2). Patients not following recommendations for maintenance treatment have an increased risk for developing major and minor motor symptoms (node 5). The majority of patients who adherence to emergency and maintenance treatment remain asymptomatic (node 4).

Fig. 3. Kidney function (GFR estimated by Schwartz formula[23]) of 54 patients at last visit. GFR declined with age, starting between childhood and adolescence. Three patients older than 12 years had GFR below 90 ml/min per 1·73 m2 (CKD stage 2). Orange line indicates linear regression fit, blue dotted line indicates scatter plot smoother. Patients with high (+) and low excreting (-) phenotype did not differ.

Fig. 4. Survival of patients over time using Kaplan-Meier analysis. Asymptomatic patients do not differ from patients with mild or moderate movement disorder and show the best survival rate. In contrast, patients with severe movement disorder have a lower life expectancy than other patients ($p<0.001$). Cross-marks indicate censored patients.



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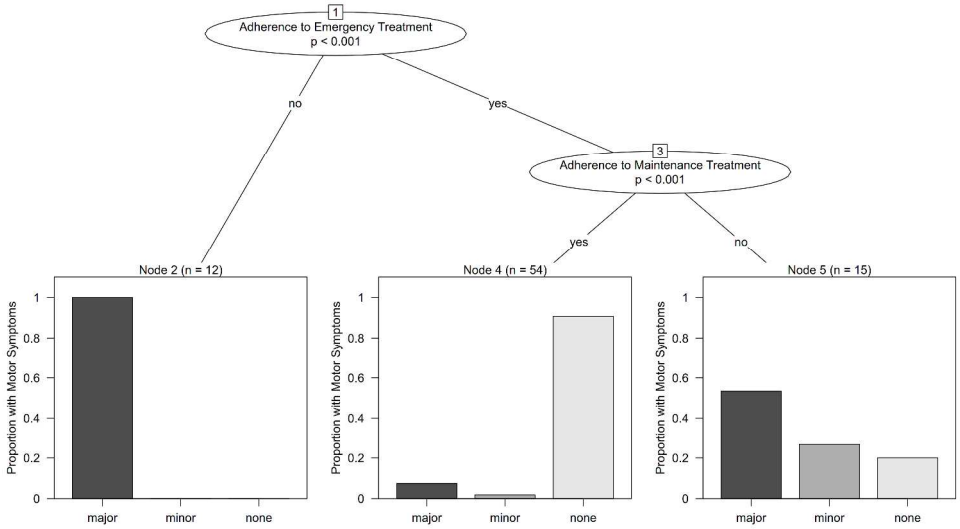

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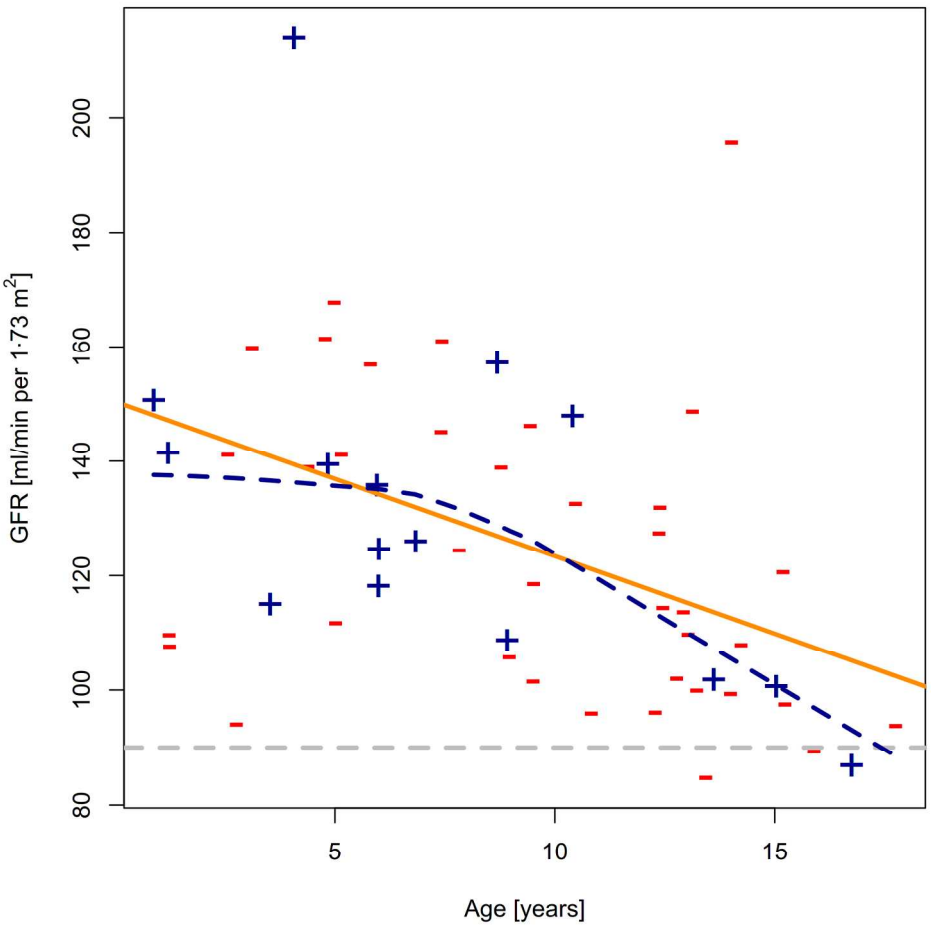

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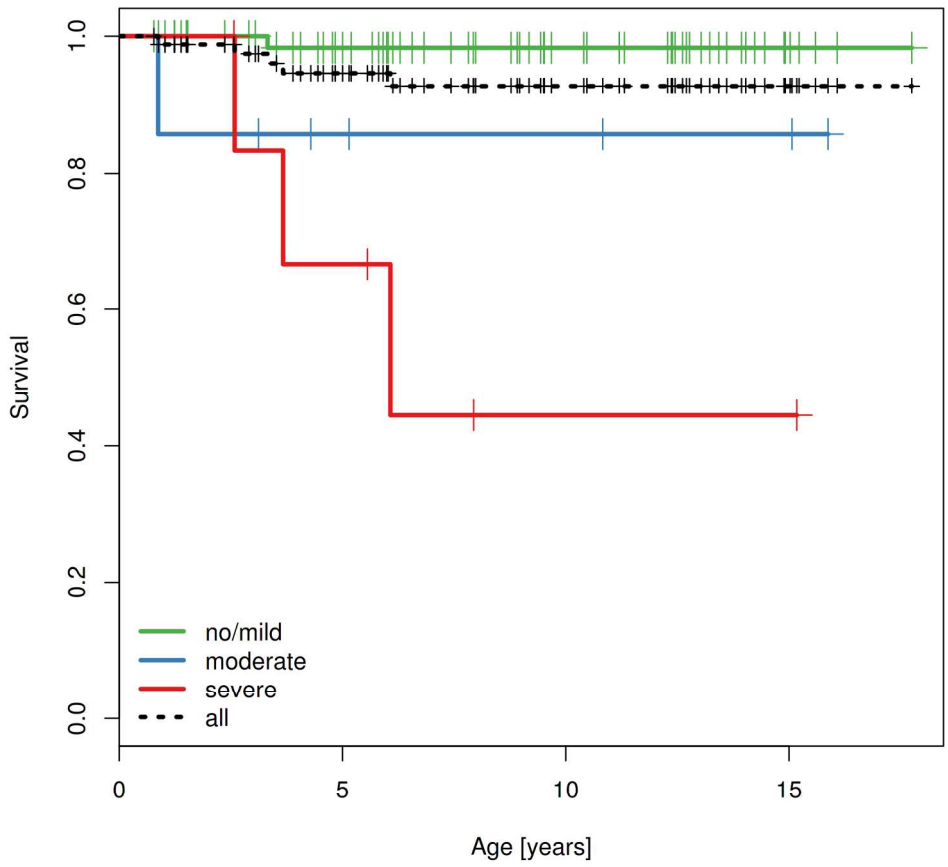

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