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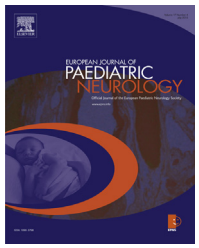

Original article

Glutaric aciduria type I: Outcome of patients with early- versus late-diagnosis

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ABSTRACT

Patients with Glutaric aciduria type 1 (GA-1) can be identified by newborn screening using tandem mass spectrometry. The clinical evolution of screened patients seems to be more favourable compared with those diagnosed later, although long-term evolution is still doubtful. We have evaluated the outcome in nine GA-1 patients diagnosed in our region during 12 years. Six were detected by newborn screening and 3 clinically. The birth prevalence was 1:35,027. High blood C5DC concentration, in 8/9 patients, was found, whereas all patients exhibited high concentration of this metabolite in urine. Therefore, urine C5DC was a good marker for the detection of this disease. Eight different mutations in the *GCDH* gene were identified, four of them were novel (p.R88H, p.Y398C, p.R372K, p.D220N); being p.R227P the mostcommon. Macrocephaly with enlarged frontotemporal subarachnoid space was present in 4/6 patients diagnosed by newborn screening, all these patients required high energy intake, and in two cases, enteral feeding during the first year of life was needed. One child had an intercurrent episode of feeding refuse with hypoglycemia at two years of age. The mean follow-up time of screened patients was 56 months, and patients still remain asymptomatic. However, after a mean follow-up of 97 months treatment efficacy was poor in unscreened patients, two of them showing a severe spastic tetraparesis. Plasma levels of lysine, tryptophan and carnitine, were the most useful biomarkers for the follow-up.

Our data support that, early diagnosis and treatment strategies are essential measures for the good clinical evolution of GA-1 patients.

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1. Introduction

Glutaric aciduria type I (GA-1, OMIM 231669) is a rare metabolic disorder of autosomal recessive inheritance caused by deficient or nonfunctional glutaryl-CoA dehydrogenase (GCDH, EC 1.3.99.7) which acts in the metabolic pathway of lysine, hydroxylysine and tryptophan.[1–5] An estimated prevalence of 1 in 100,000 newborns has been found.[6] However, in certain communities there is an over-expression of this entity, such as the Amish Community,[4,7] the Canadian Oji-Cree natives[4,8] and the Lumbee in North Carolina.[4] In Galicia (North-East of Spain), the reported incidence was 1 in 35,027 newborns.[9]

The human *GCDH* gene is localized on chromosome 19p13.2; and more than 200 disease-causing mutations are known.[10–13] The metabolic block leads to an accumulation of glutarylcarnitine (C5DC), glutaric (GA) acid and 3-hydroxyglutaric acid (3-OH-GA) in body fluids. The diagnosis is usually established by measuring these metabolites in urine. However, some GA-1 patients are low excretors, they only show moderately abnormal excretion of 3-OH-GA with normal or a slight increase GA.[3,9,13] In these cases, molecular study or measurement of the enzymatic activity are necessary to establish the diagnosis.

Clinical onset of the disease usually occurs in early childhood (between 3 and 24 months of age) as an acute encephalopathic crisis which produces necrosis of the basal ganglia leading to an important neurological dysfunction resembling an episode of encephalitis with movement disorders such as dystonia and/or dyskinesia.[3,13,14]

Measurement of C5DC together with the ratios C5DC/C16, C5DC/C5OH, C5DC/C8 by tandem mass spectrometry (MS/MS) in dried blood spots allows the pre-symptomatic detection of GA1 in newborns and consequently, early treatment can be administered.[15–17] In some newborn screening (NBS) centres the parallel measurement of C5DC, GA and 3-OH-GA in dried urine spots is a helpful tool to increase the specificity of the screening procedure.[9,18,19] The clinical evolution of patients detected by NBS seems to be favourable in most cases, although long-term evolution is still doubtful.[20–23] We present the follow up data of the children with GA-1 diagnosed in Galicia in the last 12 years, either by NBS or by clinical symptoms.

2. Patients and methods

Children with GA-1 detected between June 1999 and May 2011 in Galicia were studied in the Metabolic Disease and Neurology Units of our Hospital. All the children diagnosed both, symptomatically and by NBS were included in the present study.

2.1. Evaluation of patients at diagnosis

At diagnosis the following parameters were evaluated: age, sex, anthropometric parameters, diagnostic method (NBS or late-diagnosis), presence or absence of clinical symptoms, imaging (cerebral US and/or cerebral MR), blood acylcarnitines, urinary C5DC and organic acid profile by MS/MS or GC-MS. The diagnosis was confirmed in all cases by mutation analysis of the *GCDH* gene.

In late-diagnosed cases, the neonatal screening samples were recovered and analyzed retrospectively (samples were keep frozen at −20 °C).

2.2. Patients' follow-up

Once established the diagnosis, all patients received a dietary restriction of lysine and tryptophan, patients older than 6 years of age received a less restrictive diet. To avoid potential nutritional deficiencies we used a computer programme of dietary calculation developed by ourselves that monitors natural and total proteins, lysine, tryptophan and energy intake (www.odimet.es).

Patients also received an initial oral dosage of 100 mg/kg per day of L-carnitine divided in 3–4 doses, later on adjusted to maintain the concentration of L-carnitine within the normal range. Riboflavin (100 mg per day) was also administered. Unscreened patients also required other drugs such us: GABA agonist, benzodiazepines, antiepileptic drugs and botulinum toxin.

Aggressive treatment of the intercurrent infections was done by cessation or reduction of protein intake to 50% for 24 h or less, depending on the severity of the illness, whilst providing a high energy intake with an extra 20% of caloric requirements through carbohydrates, lipids and double dose of carnitine.

Biochemical follow-up included periodic monitoring of blood free carnitine and acylcarnitines, as well as plasma aminoacids including lysine and tryptophan.

Anthropometric evaluation was done by measuring body weight, body length and head circumference, expressed as percentiles with respect to the reference population.

Neurological assessment was based on the scoring system of Kyllerman et al.[24] Cognitive function was assessed by Psychomotor Development Index (PDI) or Intellectual Quotient (IQ) using the Brunet Lézine Scale in infants, the McCarthy Scales of Psychomotor Skills (MSCA) in preschool children and Wechsler Intelligence Scale for Children Revised (WISC-R) in children older than 6 years. The overall index score of PDI or IQ is considered in the normal range when it is above 85.

2.3. Analytical methods

The NBS laboratory simultaneously receives blood and urine samples impregnated on Whatman 903 paper from every newborn. Acylcarnitines, including C5DC, were analyzed as butyl ester derivatives by MS/MS in dried blood spots taken on the third day of life. When blood C5DC concentration was elevated (cut off $\leq$ 0.13 μM since 2007 until now; $\leq$0.15 μM since 2000–2006), a dried urine spot was analyzed selectively to determine C5DC, 3-OH-GA and GA by MS/MS.[25]

The percentile of each blood metabolite obtained by MS/MS in healthy neonatal population, as well as those pathologies detected through the NBS programme, are periodically reported to Region 4 Genetics collaborative Project,[26] achieving clinical validation of our cutoff values. On the other hand,

control values for the different metabolites in dried urine spots in the neonatal population were obtained in a sample of 3000 neonates and percentile was also used to express the results. Monitoring of plasma amino acids was carried out by ion exchange chromatography (*Biochrom 30* analyzer). Urine organic acids were analyzed by MS/MS when using dried spots and by gas chromatography/mass spectrometry in case of liquid samples.

Mutation analysis of the *GCDH* gene were done by Sanger sequencing of all exons and flanking intronic regions.

Informed consent of the patients' parents was obtained. The study was approved by our Hospital's Ethics Committee.

3. Results

During the 12-year study period 248,415 newborns were screened, 6 patients were positive by NBS, 1 patient was a false negative and 1 patient, born during this period, was not subjected to NBS. Therefore, the incidence of GA1 in our region is 8 in 248,415 or 1 in 31,052. Consanguinity was denied by all families and all of them were Caucasian.

Patients (p1 to p6) detected by NBS (Table 1), presented a high concentration of C5DC in blood spots (mean: 1.65 μM; range: 0.27–4.23; cutoff ≤ 0.13 μM). Urine C5DC resulted clearly increased in all them (mean: 49.7 mmol/mol creatinine; range: 14.5–132; control values: <1.9), 3-OH-GA was also increased in all the patients, while glutaric acid was only elevated in 4/6 patients (Table 1). All of them were asymptomatic at diagnosis although 4 (patients 2, 4, 5, 6) presented macrocephaly, birth head circumference was 37.6 cm (36.7–38.1 cm) (>90th percentile); weight and length was in normal percentiles; and enlarged frontotemporal subarachnoid spaces in the imaging (MRI and/or cerebral US). Treatment consisted of carnitine supplementation, riboflavin (during the first two years of life) and moderate dietetic restriction of lysine. During infectious processes or situations of catabolic stress a strict treatment was set up in all patients diagnosed by NBS (Table 2). Interestingly, during the first year of life all of them frequently needed high energy intake. To maintain an adequate nutrition and weight percentile in a proper range for their age, two patients (p5 and p6) also required partial enteral feeding during 5 and 4 months, respectively. Both presented with the highest values of potentially toxic metabolites (Table 1). During the follow-up plasma levels of tryptophan and lysine were maintained in the appropriate range, with lysine sometimes even low. Free carnitine levels remained over 30 μM in all cases; high blood C5DC levels persisted in all cases except in p3. Urinary concentrations of C5DC was persistently elevated in all cases; GA and 3-OH-GA were increased in 4 of them (p2, p3, p5, p6), (data not shown). One of the children (p2) presented with a severe intercurrent episode of refusal to feed with hypoglycemia at two years of age. The mean follow up time was of 56 months (from 16 months to 9 years and 9 months) and all patients still remain asymptomatic, with a normal motor, spoken and cognitive level; the initial macrocephaly persists and one patient (p2) was diagnosed of attention deficit at six years of age (Table 2).

Three patients (p7–p9) were diagnosed clinically. Two of them (p7 and p8) showed an acute encephalopathy (Table 1). Brain MRI and clinical symptoms both were consistent with GA-1. One of these patients (p7) was a false negative of the NBS programme because blood glutarylcarnitine concentration was 0.13 μM (99.9 percentile of healthy neonatal population: 0.18 μM, cut off 0.15 μM at that date) but retrospective analysis of glutarylcarnitine in the patient's neonatal urine spot was high: 18.2 mmol/mol creatinine (cutoff: <1.9), as well as the concentration of 3-OH-GA: 204 mmol/mol creatinine (cutoff < 47) and of GA: 127 mmol/mol creatinine (cutoff < 109). Clinical symptoms in this patient started at 8 months of age, after an acute encephalopathy. The third patient (p9) had two previous episodes of decompensation (at 13 months of age, when symptoms started, and at 4 years of age)that were labeled as probable viral encephalopathy. At diagnosis he showed a severe dystonic spastic tetraparesis and seizures (Table 1). The mean follow up time of these three patients was 97 months. Despite dietetic treatment and pharmacological supplements of riboflavin, L-carnitine, GABA agonist and benzodiazepines (as well as antiepileptic therapy and botulinum toxin in p9) clinical evolution was very poor in p7 and p9 (Table 2) and they were considered as PDI/IQ <60. However, patient p8 that presented an episode of encephalitis at 14 months of age, showed a good recovery with normal spoken and cognitive levels at 11 years of age. He only shows isolated upper extremity dystonias. The parameters of developmental control are maintained at a normal range.

All mutations are summarized in Table 1. Among 18 alleles (9 cases) eight different mutations were found, p.R227P being the most frequently identified (28%of the alleles). Four mutations were novel: mutation p.R88H (c.263G > A) was present in homozygosity in 2 cases diagnosed by screening; mutation p.Y398C (c.1229A > G) was detected in homozygosity in one case and in heterozygosity in another one; mutations p.R372K (c.1151G > A) and p.D220N (c.694G > A), both were identified in heterozygosity in one patient. All the new mutation were predicted as probably damaging by PolyPhen data base (http://coot.embl.de/PolyPhen), and were not found in more than 200 control alleles. Homozygosity was detected in 4/9 cases. However, there was no demonstrated parents' consanguinity.

4. Discussion

The prevalence of GA-1 in our region is far superior to that found in the medical literature, independently if it was found through NBS or deduced through other sources.[6,20,21,27] Predictably, as alreadyshown in other studies[6,20–23] and as already published by us,[28] early detection and treatment of this condition before the classical onset of encephalitis, leads to a major positive change in the natural history of GA-1.

However, diagnosis through NBS is not always evident. If patients are low excretors blood C5DC could be within the reference range,[18] as it was the case in our patient with a false negative result in 2006 and as described before by other authors.[29,30] Urine C5DC or urine 3-OH-GA had been described as excellent biomarkers for this disease.[18,19] In our hands, urine C5DC was a good biomarker, as all patients showed high levels of this metabolite at least 3.7 times over the control range. Since 2004 we have screened for C5DC in urine as a second tier test. However, in order to avoid false negative

Table 1 – Clinical and biochemical data of patients affected with glutaric aciduria type 1 at diagnosis.

Patient	1	2	3	4	5	6	7	8	9
Sex	M	F	M	F	F	F	M	M	M
Age at diagnosis	11 days	19 days	15 days	20 days	11 days	18 days	8 months	14 months	13 y 10 m
Type of diagnosis	NBS	NBS	NBS	NBS	NBS	NBS	CS	CS	CS
CS at diagnosis	None	Macrocephaly	None	Macrocephaly	Macrocephaly	Macrocephaly	Encephalopathy	Encephalopathy	Spastic tetraparesis. Seizures.
C5DC in blood spots (cutoff $\leq$ 0.13 μM)	0.48	0.58	0.53	0.27	3.86	4.23	0.20	0.25	1.66
C5DC in urine (cutoff < 1.9 mmol/mol creatinine)	43.1	17.5	15.9	14.5	132.0	68.0	7.2	13.8	ND
3-Hydroxyglutaric acid in urine[†]	238	1267	70.0[a]	176.3[a]	368[a]	574[a]	18	70	Increased
Glutaric acid in urine[‡]	412	86	82.0[b]	40[b]	5160[b]	3509[b]	17	23	177
Molecular studies:									
Nucleotide change	c.716G > C c.950C > T	c.298C > T c.1229A > G	c.694G > A c.1240C > T	c.716G > C c.716G > C	c.299G > A c.299G > A	c.299G > A c.299G > A	c.716G > C c.716G > C	c.1229A > G c.1229A > G	c.1151G > A c.1240C > T
Effect on protein	p.R227P/p.S305L	p. R88C/p.Y398C	p.D220N/p.R402W	p.R227P/p.R227P	p.R88H/p.R88H	p.R88H/p.R88H	p.R227P/p.R227P	p.Y398C/p.Y398C	p.R372K/p.R402W
Imaging at diagnosis	N	A	N	A	A	A	A,B,C	A,B	A,C,D

M: male; F: female; NBS: newborn screening; CS: clinical symptoms; C5DC: glutarylcarnitine; CV: control values; ND: no done.

[†]Control values: 2–15 mmol/mol creatinine; [a]urine spots, cutoff < 46.7 mmol/mol creatinine.

[‡]Control values: 2–10 mmol/mol creatinine; [b]urine spots, cutoff < 109.3 mmol/mol creatinine.

N: normal, A: enlarged subarachnoid frontotemporal spaces with sylvian fissures opening in relation to selective atrophy in the region; B: hyperintensity of basal ganglia in T2-weighted images; C: bilateral cerebral demyelination; D: diffuse cerebral atrophy.

Table 2 – Follow-up data of patients diagnosed of GA-1.

	1	2	3	4	5	6	7	8	9
Actual age	9 y 9 m	9 y 7 m	3 y 6 m	2 y 2 m	23 m	16 m	4 y 9 m	11 y 11 m	23 y 4 m
Type of diagnosis	NBS	NBS	NBS	NBS	NBS	NBS	CS	CS	CS
Symptoms	Asymptomatic (head circ. 55.5 cm)	Attention deficit	Asymptomatic	Asymptomatic Macrocephaly	Asymptomatic Macrocephaly	Asymptomatic Macrocephaly	Dystonic spastic tetraparesis. Choreoathetosis.	Isolated dystonic movements.	Severe spastic tetraparesis. Seizures.
Treatment	L-carnitine	L-carnitine	L-carnitine	L-carnitine	L-carnitine, B2	L-carnitine, B2	L-carnitine, B2, GABA agonist	L-carnitine, GABA agonist	L-carnitine, GABA agonist, antiepileptic, Botulinum toxin
Dietary treatment	Lys–Try restricted + emergency treatment of intercurrent illness	Lys–Try restricted + emergency treatment of intercurrent illness	Lys–Try restricted + emergency treatment of intercurrent illness	Lys–Try restricted + emergency treatment of intercurrent illness	Lys–Try restricted + emergency treatment of intercurrent illness	Lys–Try restricted + emergency treatment of intercurrent illness	Lys–Try restricted + emergency treatment of intercurrent illness	Moderate Lys–Try restriction	Slightly Lys–Try restriction
Lysine in plasma: Mean (range) RV: 77–181 μM	72.8 (37–102)	85.6 (34.8–173)	40 (23.8–68.1)	81.6 (44.4–120)	89.1 (61.1–139)	58.9 (19.9–92)	79.1 (18.6–129)	76.4 (37.8–124)	107.3 (79.9–179)
Tryptophan in plasma: Mean (range) RV: 5–57 μM	41.9 (35.7–49.3)	48.5 (33.1–86.6)	46.7 (23.1–70.7)	49.2 (38.9–60.8)	41.5 (31.8–48.8)	45.4 (28.1–59.8)	28.6 (5.7–51.5)	41.9 (21.3–58.3)	41.7 (21.2–73)
Free carnitine in blood spots: Mean (range) RV: 6–55 μM	41.4 (34.0–59.0)	50.4 (41.7–60.0)	66.1 (49.3–93.2)	60.6 (35.9–122)	65.2 (43.5–82.2)	74.8 (51.3–93.0)	52.4 (31.2–71.8)	43.0 (40.3–50.5)	61.1 (39.0–81.0)
C5DC in blood spots: Mean (range) Cutoff $\leq$ 0.13 μM	0.20 (0.14–0.29)	0.80 (0.56–0.92)	0.10 (0.03–0.28)	0.20 (0–0.44)	5.5 (3.60–8.09)	3.5 (1.84–7.18)	0.10 (0.04–0.19)	0.40 (0.23–0.56)	2.3 (1.2–3.33)
Motor disability[a]	A	A	A	A	A	A	B	A	B
Cognitive function	105	85	95	100	110	100	<60	99	<60
Speech and language	Fluent	Fluent	Fluent	Fluent	Fluent	Fluent	No speech	Nearly fluent	No speech

NBS: newborn screening. CS: clinical symptoms. C5DC: glutarylcarnitine. RV: reference values. Lys: lysine. Try: tryptophan.
a A: without or only slight motor dysfunction with no disability in daily life; B: wheelchair dependency and severe disability in daily life.

results it will be appropriate to analyze C5DC in all newborn urine spots sent to our NBS program.

The frequency of macrocephaly (66.6%) in presymptomatic patients is similar to other reports (65–75%).[20,31] We have not found any significant correlation between macrocephaly and the clinical outcome but, interestingly, it correlates with the feeding difficulties in the first year of life. Patients detected by NBS presenting macrocephaly at the time of diagnosis, needed higher energy intake, and two of them even needed enteral feeding for a few months to reach an age-appropriate weight. Toxic metabolites probably start accumulating in utero[21] and for a still unknown reason some patients might be more vulnerable than others.

Carnitine status and plasma amino acid concentrations are the major biochemical parameters monitored in these patients.[5,22,32,33] As it has been reported previously[14,34] urine C5DC, GA and 3-OH-GA did not show any correlation with the clinical evolution. Therefore, measurements of these parameters were discontinued.

Initiation of treatment after the onset of symptoms was not effective in preventing permanent neurological injuries. However, treatment may be beneficial for the prevention of the progressive neurological deterioration[13,14,24,31] and even more, in some cases it might be a factor contributing to the clinical improvement, as it happened in our patient p8 who showed a complete recovery of the motor function including speech, and at present he only shows mild extrapyramidal symptoms. Despite striatal injury occurs acutely or insidiously until age 6 years; and despite there is evidence for ongoing extra-striatal neuroradiological changes after age 6 years[35] of doubtful clinical relevance it may be advisable to continue dietary treatment using a less stringent protocol than that used prior to age 6 years,[5] as it was done in our patients. As there is no firm evidence that riboflavin improves the neurological outcome of this disease[5] and it didn't seem to influence the outcome of our patients, this therapy was withdrawn in our diagnosed screening patients at two years of life.

It has been described that the high-excretor phenotype was associated with mutations that resulted in very low or null enzymatic activity, while the low-excretor phenotype was associated with residual activity, but a correlation with the clinical phenotype could not be established.[13,14,34] Like these authors, we could not establish any correlation genotype–clinical phenotype. In our study, we found that low-excretors have the same risk of developing striatal injury as high excretors; in fact, one of the low-excretors, diagnosed at 8 months of age, developed a very unfavorable disease with spastic tetraparesis.

It has been reported that R402W is the most common mutation in Caucasians,[11,13,34,36] but in our study it was only found in 2 alleles, while R227P was found in 8 alleles, being the most prevalent mutation in our population (28% of the alleles). Interestingly, the prevalence of this mutation was similar to that found in a large study of Spanish[13] and other European[34] patients, but it has never been found in homozygosity, while two homozygous patients were found in the present study. As expected, homozygous patients for mutation p.R227P follow the low-excretor pattern, but it was not expected that patient 1, compound heterozygous for this and for mutation p.S305L, follows the high-excretor pattern. Mutation p.Y398C described here for the first time, should be added the list of mutations causing low excretion, both when found in homozygosity (p8) or in compound heterozygosity (p2). It is interesting to note that mutation p.R88H, also described here for the first time, causes the same biochemical phenotype than the previously described mutation, p.R88C, in the same protein position.[11] In conclusion, GA-1 is relatively frequent in our region. The number of high and low excretors is very similar, therefore measurement of C5DC in urine spots seems very promising to avoid false negative results in NBS. Early treatment gives rise to a normal neurological outcome. Nevertheless, in patients that have already developed macrocephaly at the time of diagnosis, a higher than usual energy intake is required to achieve sufficient growth in the first year of life.

REFERENCES

1. Goodman SI. Organic acidemias due to defects in lysine oxidation: 2-ketoadipic acidemia and glutaric acidemia. In: Scriver ea, editor. *Mol Met Basis Inher Dis*. New York: McGraw-Hill Inc; 2001. p. 2195–204.
2. Goodman SI, Markey SP, Moe PG, Miles BS, Teng CC. Glutaric aciduria; a "new" disorder of amino acid metabolism. *Biochem Med* 1975;**12**:12–21.
3. 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. Guideline for the diagnosis and management of glutaryl-CoA dehydrogenase deficiency (glutaric aciduria type I). *J Inherit Metab Dis* 2007;**30**:5–22.
4. Kölker S, Christensen E, Leonard JV, Greenberg CR, Boneh A, Burlina AB, Burlina AP, Dixon M, Duran M, García Cazorla A, Goodman SI, Koeller DM, Kyllerman M, Mühlhausen C, Müller E, Okun JG, Wilcken B, Hoffmann GF, Burgard P. Diagnosis and management of glutaric aciduria type I – revised recommendations. *J Inherit Metab Dis* 2011;**34**:677–94.
5. Mushimoto Y, Fukuda S, Hasegawa Y, Kobayashi H, Purevsuren J, Li H, Taketani T, Yamaguchi S. Clinical and molecular investigation of 19 Japanese cases of glutaric acidemia type 1. *Mol Genet Metab* 2011;**102**:343–8.
6. Lindner M, Kölker S, Schulze A, Christensen E, Greenberg CR, Hoffman GF. Neonatal screening for glutaryl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 2004;**27**:851–9.
7. Morton DH, Bennett MJ, Seargeant LE, Nichter CA, Kelley RI. Glutaric aciduria type I: a common cause of episodic encephalopathy and spastic paralysis in the Amish of Lancaster County, Pennsylvania. *Am J Med Genet* 1991;**41**:89–95.
8. Haworth JC, Dilling LA, Seargeant LE. Increased prevalence of hereditary metabolic diseases among native Indians in Manitoba and northwestern Ontario. *Can Med Assoc J* 1991;**2**:123–9.
9. Couce ML, Castiñeiras DE, Bóveda MD, Baña A, Cocho JA, Iglesias AJ, Colón C, Alonso-Fernández JR, Fraga JM. Evaluation and long-term follow-up of infants with inborn errors of metabolism identified in an expanded screening programme. *Mol Genet Metab* 2011;**104**:470–5.
10. Goodman SI, Stein DE, Schlesinger S, Christensen E, Schwartz M, Greenberg CR, Elpeleg ON. Glutaryl-CoA dehydrogenase mutations in glutaric academia (type I): review and report of thirty novel mutations. *Hum Mutat* 1998;**12**:141–4.

11. Zschocke J, Quak E, Guldberg P, Hoffmann GF. Mutation analysis in glutaric aciduria type I. *J Med Genet* 2000;**37**:177–81.
12. Schwartz M, Christensen E, Superti-Fuga A, Brandt NJ. The human glutayl-CoA dehydrogenase gene: report of intronic sequences and of 13 novel mutations causing glutaric aciduria type I. *Hum Genet* 1998;**102**:452–8.
13. Busquets C, Merinero B, Christensen E, Gelpí JL, Campistol J, Pineda M, Fernández-Alvarez F, Prats JM, Sans A, Arteaga R, Martí M, Campos J, Martínez-Pardo M, Martínez-Bermejo A, Ruiz-Falcó ML, Vaquerizo J, Orozco M, Ugarte M, Coll MJ, Ribes A. Glutaryl-CoA dehydrogenase deficiency in Spain: evidence of two groups of patients, genetically, and biochemically distinct. *Pediatr Res* 2000;**48**:315–22.
14. 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, Martí-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. Natural history, outcome, and treatment efficacy in children and adults with glutaryl-CoA dehydrogenase deficiency. *Pediatr Res* 2006;**59**:840–7.
15. Lindner M, Ho S, Fang-Hoffmann J, Hoffmann GF, Kölker S. Neonatal screening for glutaric aciduria type I: strategies to proceed. *J Inherit Metab Dis* 2006;**29**:378–82.
16. Chace DH, Kalas TA, Naylor EW. Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns. *Clin Chem* 2003;**49**:1797–817.
17. Wilcken B, Haas M, Joy P, Wiley V, Bowling F, Carpenter K, Christodoulou J, Cowley D, Ellaway C, Fletcher J, Kirk EP, Lewis B, McGill J, Peters H, Pitt J, Ranieri E, Yaplito-Lee J, Boneh A. Expanded newborn screening: outcome in screened and unscreened patients at age 6 years. *Pediatrics* 2009;**124**:e241–8.
18. Tortorelli S, Hahn SH, Cowan TM, Brewster TG, Rinaldo P, Matern D. The urinary excretion of glutarylcarnitine is an informative tool in the biochemical diagnosis of glutaric acidemia type I. *Mol Genet Metab* 2005;**84**:147–53.
19. Al-Dirbashi OY, Kölker S, Ng D, Fisher L, Rupar T, Lepage N, Rashed MS, Santa T, Goodman SI, Geraghty MT, Zschocke J, Christensen E, Hoffmann GF, Chakraborty P. Diagnosis of glutaric aciduria type 1 by measuring 3-hydroxyglutaric acid in dried urine spots by liquid chromatography tandem mass spectrometry. *J Inherit Metab Dis* 2011;**34**:173–80.
20. Bijarnia S, Wiley V, Carpenter K, Christodoulou J, Ellaway CJ, Wilcken B. Glutaric aciduria type I: outcome following detection by newborn screening. *J Inherit Metab Dis* 2008;**31**:503–7.
21. Boneh A, Beauchamp M, Humphrey M, Watkins J, Peters H, Yaplito-Lee J. Newborn screening for glutaric aciduria type I in Victoria: treatment and outcome. *Mol Genet Metab* 2008;**94**:287–91.
22. Heringer J, Boy SP, Ensenauer R, Assmann B, Zschocke J, Harting I, Lücke T, Maier EM, Mühlhausen C, Haege G, Hoffmann GF, Burgard P, Kölker S. Use of guidelines improves the neurological outcome in glutaric aciduria type I. *Ann Neurol* 2010;**68**:743–52.
23. Kölker S, Garbade SF, Boy N, Maier EM, Meissner T, Mühlhausen C, Hennermann JB, Lücke T, Häberle J, Baumkötter J, Haller W, Muller E, Zschocke J, Burgard P, Hoffmann GF. Decline of acute encephalopathic crises in children with glutaryl-CoA dehydrogenase deficiency identified by newborn screening in Germany. *Pediatr Res* 2007;**62**:357–63.
24. Kyllerman M, Skjeldal O, Christensen E, Hagberg G, Holme E, Lönnquist T, Skov L, Rotwelt T, von Döbeln U. Long-term follow up, neurological outcome and survival rate in 28 Nordic patients with glutaric academia type 1. *Eur J Paediatr Neurol* 2004;**8**:121–9.
25. Rebollido M, Cocho JA, Castiñeiras DE, Bóveda MD, Fraga JM. Aplicación de la espectrometría de masas en tándem al análisis de aminoacidos, acilcarnitinas, acilglicinas y ácidos orgánicos en muestras de orina en papel. *Quím Clin* 2006;**25**:64–74.
26. McHugh DM, Cameron CA, Abdenur JE, Abdulrahman M, Adair O, Al Nuaimi SA, et al. Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: a worldwide collaborative project. *Genet Med* 2011;**13**:230–54.
27. Niu DM, Chien YH, Chiang CC, Ho HC, Hwu WL, Kao SM, Chiang SH, Kao CH, Liu TT, Chiang H, Hsiao KJ. Nationwide survey of extended newborn screening by tandem mass spectrometry in Taiwan. *J Inherit Metab Dis* 2010;**33**:S295–305.
28. Couce Pico ML, Castiñeiras Ramos DE, López Sousa M, Fernández Seara MJ, Eirís Puñal J, Cocho de Juan JA. Importance of early diagnosis and treatment in the prognosis of type I glutaric acidaemia. *An Pediatr* 2008;**69**:239–43.
29. 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. *Mol Genet Metab* 2005;**86**:417–20.
30. Wilcken B, Wiley V, Hammond J, Carpenter K. Screening newborns for inborn errors of metabolism by tandem mass spectrometry. *N Engl J Med* 2003;**348**:2304–12.
31. Bjugstad KB, Goodman SI, Freed CR. Age at symptom onset predicts severity of motor impairment and clinical outcome of glutaric acidemia type 1. *J Pediatr* 2000;**137**:681–6.
32. Müller E, Kölker S. Reduction of lysine intake while avoiding malnutrition – major goals and major problems in dietary treatment of glutaryl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 2004;**27**:903–10.
33. Seccombe DW, James L, Booth F. L-carnitine treatment in glutaric aciduria type I. *Neurology* 1986;**36**:264–7.
34. Christensen E, Ribes A, Merinero B, Zschocke J. Correlation of genotype and phenotype in glutaryl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 2004;**27**:861–8.
35. Harting I, Neumaier-Probst E, Seitz A, Maier EM, Assmann B, Baric I, Troncoso M, Mühlhausen C, Zschocke J, Boy NP, Hoffmann GF, Garbade SF, Kölker S. Dynamic changes of striatal and extrastriatal abnormalities in glutaric aciduria type I. *Brain* 2009;**132**:1764–82.
36. Busquets C, Soriano M, de Almeida IT, Garavaglia B, Rimoldi M, Rivera I, Uziel G, Cabral A, Coll MJ, Ribes A. Mutation analysis of the GCDH gene in Italian and Portuguese patients with glutaric aciduria type I. *Mol Genet Metab* 2000;**71**:535–7.