J Inherit Metab Dis (2009) 32 (Suppl 1):S355–S359
DOI 10.1007/s10545-009-9017-6

RESEARCH REPORT

False-positive newborn screening mimicking glutaric aciduria type I in infants with renal insufficiency

Julia B. Hennermann · Sylvia Roloff · Jutta Gellermann · Annette Grüters · Jeannette Klein

Received: 16 August 2009 / Revised: 6 November 2009 / Accepted: 10 November 2009 / Published online: 23 December 2009

Abstract Glutaric aciduria type I (GA I), an autosomal-recessive deficiency of glutaryl-CoA-dehydrogenase, leads to encephalopathic crises resulting in irreversible neurological damage. As early diagnosis and implementation of appropriate treatment has significant benefit for these patients, GA I has been implemented in the extended newborn screening program in several countries. Screening parameter is glutarylcarnitine (C5DC) with its ratios. From 1 January 2005 until 31 December 2008, 173,846 newborns were examined by neonatal screening in our screening center. C5DC and/or at least three C5DC/acylcarnitine ratios were increased in 53 newborns (0.03%) and persisted in 11 infants after recall. GA I was not confirmed in any of these infants, but all 11 infants were suffering from renal insufficiency due to congenital (5/11) or acquired (6/11) renal disease. C5DC was shown to be significantly associated with renal affection and was significantly higher in infants with congenital renal insufficiency than in those with acquired renal insufficiency (p=0.011). Creatinine correlated significantly with C5DC (p=0.001) and all C5DC/acylcarnitine ratios, mainly with C5DC/(C8 + C10), C5DC/C0, C5DC/C2, C5DC/C4, and C5DC/C8 (for all: p=0.001). Glutarylcarnitinemia associated with renal insufficiency has not yet been studied systematically. Renal damage in neonates might lead to disturbances in renal transporter systems of glutaric acid and its metabolites and a decreased excretion of C5DC, thus resulting in an increase of plasma C5DC. Therefore, newborns presenting with a positive screening indicating GA I may be considered not only to suffer from GA I but from renal insufficiency as well.

Communicated by: Georg Hoffmann

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

J. Gellermann
Department of Pediatric Nephrology,
Charité Universitätsmedizin Berlin,
Berlin, Germany

A. Grüters
Institute for Experimental Pediatric Endocrinology,
Charité Universitätsmedizin Berlin,
Berlin, Germany

Abbreviations

C0	Free carnitine
C10-OH	Hydroxydecanoylcarnitine
C12	Dodecanoylcarnitine
C16	Palmitoylcarnitine
C2	Acetylcarnitine
C4	Butyrylcarnitine
C5DC	Glutarylcarnitine
C8	Octanoylcarnitine
GA I	Glutaric aciduria type I
GCDH	Glutaryl-CoA dehydrogenase
MADD	Multiple acyl-CoA dehydrogenase deficiency
MCADD	Medium-chain acyl-CoA dehydrogenase deficiency

Introduction

Glutaric aciduria type I (GA I, OMIM 231670) is an autosomal-recessive disorder caused by a deficiency of mitochondrial glutaryl-CoA dehydrogenase (GCDH, EC 1.3.99.7). The estimated overall prevalence of GA I is 1 in 100,000 newborns (Lindner et al. 2004). Mainly in catabolic situations, patients with GA I suffer from acute encephalopathic crises resulting in severe irreversible

neurological damage, dominated by movement disorders and seizures. Early diagnosis by newborn screening and early implementation of medical and dietary treatment has significant benefit for patients with GA I (Kölker et al. 2007a, b). Therefore, GA I has been implemented in the extended newborn screening program in some countries, as in Austria, Denmark, Germany, parts of the USA, and Australia. Since 2005, screening for GA I has been included in the nationwide extended German neonatal screening program (German Newborn Screening Directive, www.g-ba.de). Screening parameter is the five-carbon dicarboxylic acylcarnitine glutarylcarnitine (C5DC); some screening laboratories additionally use C5DC/acylcarnitine ratios as secondary variables (Lindner et al. 2006). Initial positive screening results should be confirmed by analyses of urinary glutaric acid and 3-hydroxy-glutaric acid. Diagnosis of GA I may be further confirmed by enzymatic and molecular analyses.

An apparent increase of C5DC has also been demonstrated in patients with medium-chain acyl-CoA dehydrogenase deficiency (MCADD) caused by an increase of hydroxydecanoylcarnitine (C10-OH), carrying the same mass transition of butyl ester as C5DC and therefore resulting in "pseudoglutarylcarnitinemia" (Napolitano et al. 2004). In patients with multiple acyl-CoA dehydrogenase deficiency (MADD), C5DC may be increased, as well, but this is accompanied by an increase of other short-, medium-, and long-chain acylcarnitines. Elevated C5DC concentrations secondary to renal insufficiency have been previously mentioned in German guidelines for GA I (www.awmf.org No. 027/018) but have not yet been studied systematically.

Materials and methods

From 1 January 2005 until 31 December 2008, 173,846 neonates born in Berlin or Brandenburg were examined by neonatal screening in our screening center. Blood spots were collected on the 1st to 8th day of life (median: 2nd day of life). Neonatal screening was performed by analyses of amino acids and acylcarnitines in dried blood samples according to standardized protocols by electrospray ionization tandem mass spectrometry. Samples were extracted with methanol containing stable-isotope-labelled internal standards. After derivatization with butanoic hydrochloric acid, amino acids and acylcarnitines were analyzed by flow injection into an API 2000 triple quadrupole mass spectrometer operating in the MRM mode (Zytkovicz et al. 2001). Semiquantitative determination of C5DC was performed with reference to deuterated octanoylcarnitine (D_3-C8). The cutoff for C5DC and its ratios was set to the 99.9th centile. C5DC/acylcarnitine ratios [C5DC/C0, C5DC/C2, C5DC/C4, C5DC/C8, C5DC/C12, C5DC/C16, and C5DC/(C8 + C10)] were used to improve diagnostic sensitivity and specificity (Lindner et al. 2006). In newborns with an increase of C5DC, pseudoglutarylcarnitinemia due to increased concentrations of C10-OH, which has the same mass transition of the butyl ester, was excluded by analyzing underivatized extracts (Napolitano et al. 2004).

Analyses of organic acids in urine were performed by gas chromatography/mass spectrometry, according to established methods. Glutaric acid was quantified in the scan mode with 3-phenylbutyric acid as internal standard. In one child, enzymatic and molecular analysis for GA I was performed by E. Christensen, Kopenhagen, and by J. Zschocke, Innsbruck, formerly Heidelberg. For statistical analyses, SPSS Version 14.0 was used, performing Mann−Whitney *U* test and Pearson correlation. For graphical presentation, box blots were used.

Results

In extended newborn screening performed in our screening center over a period of 4 years, C5DC and/or at least three C5DC/acylcarnitine ratios were increased in a total of 53 out of 173,846 newborns (0.03%). On recall examination, C5DC and/or at least three C5DC/acylcarnitine ratios remained elevated in 11/53 newborns (0.006% of totally screened newborns). None of these infants showed an increase or decrease of any further acylcarnitines, and free carnitine (C0), especially, was within normal ranges. Table 1 shows the values of C5DC and its corresponding ratios in these 11 children obtained on routine newborn screening on days 2–8 (median 3rd day) of life. In healthy newborns, C5DC is only slightly age dependent, showing a maximum value on the 1st to 2nd day of life and decreasing slowly within the first week of life (personal observation).

Analyses of organic acids in urine, performed immediately after the first positive screening result, revealed normal concentrations of glutaric acid (0–2.6 mmol/mol creatinine), normal concentrations of 2-hydroxy-glutaric acid, and no detection of 3-hydroxy-glutaric acid or glutaconic acid in all children. Repeated analyses of organic acids in urine confirmed this, questioning the initial suspicion of GA I. In contrast, all 11 children with a positive newborn screening result indicating GA I were suffering from renal insufficiency: 5/11 from congenital renal insufficiency, 6/11 from acquired renal insufficiency. Furthermore, C5DC normalized in all children with acquired renal disease within 13–39 (median 26.5) days of life and in 3/5 children with congenital renal insufficiency. This decrease of C5DC was clearly correlated with normalization of serum creatinine, thus reflecting improvement of renal function. One child with congenital renal hypoplasia (ID3) died during the neonatal period and could not be followed up further. In one child with end-stage renal insufficiency, C5DC and its ratios remained elevated. Enzymatic analysis, performed by E. Christensen, Kopenhagen, and molecular analysis, performed by J.

Table 1 Values for glutarylcarnitine (C5DC), C5DC/acylcarnitine ratios, and retention parameters in infants with renal insufficiency. All parameters were taken at the time of routine newborn screening on days 2–8 (mean 3rd day) of life

Parameter	Min	Max	Median	SD	Cutoff/normal value
C0 (μmol/L)	16.02	37.64	23.60	7.56	7.7–68
C5DC (μmol/L)	0.09	0.28	0.13	0.56	<0.10
C5DC/C0 (μmol/L)	0.003	0.008	0.007	0.002	<0.005
C5DC/C2 (μmol/L)	0.000	0.010	0.007	0.003	<0.003
C5DC/C4 (μmol/L)	0.159	0.972	0.54	0.27	<0.47
C5DC/C8 (μmol/L)	0.90	2.35	1.41	0.47	<1.21
C5DC/C12 (μmol/L)	0.33	2.26	0.96	0.55	<0.66
C5DC/C16 (μmol/L)	0.035	0.129	0.08	0.029	<0.042
C5DC/(C8 + C10) (μmol/L)	0.264	0.938	0.74	0.21	<0.46
Creatinine (mg/dl)	1.10	6.02	4.0	1.39	0.3–0.7 (newborns)
Urea (mg/dl)	60	115	72.0	22.0	<37 (newborns)

Zschocke, Innsbruck, formerly Heidelberg, was suspicious of heterozygosity for glutaryl-CoA-dehydrogenase deficiency [residual enzyme activity measured in leukocytes 30%; molecular analysis heterozygosity for I83M (c.122G>C) in *GCDH*]. As after renal transplantation acylcarnitine profile completely normalized in this child, GA I could be definitively excluded.

All children suffering from renal insufficiency had a significant increase of serum creatinine [mean 3.15 mg/dl; standard deviation (SD) 1.75] and serum urea (mean 79.34 mg/dl; SD 35.84). Five infants suffered from congenital renal insufficiency due to renal dysplasia or dysgenesia, polycystic kidney disease, or hydronephrosis and six infants from acquired renal insufficiency due to perinatal asphyxia or extreme prematurity (Table 2). In infants with congenital renal insufficiency, C5DC was significantly higher than in those with acquired renal insufficiency (p=0.011) (Fig. 1). Concentrations of C5DC and serum creatinine correlated significantly (p=0.001) (Fig. 2). Furthermore, all C5DC/acylcarnitine ratios correlated significantly with serum creatinine, but the strongest associations were found for C5DC/(C8 + C10), C5DC/C0, C5DC/C2, C5DC/C4, and C5DC/C8 (for all: p=0.001). Less significant were the correlations of creatinine with C5DC/C12 (p=0.005),and C5DC/C16 (p=0.090). However C5DC/C16 was increased in 10/11 patients, partly due to the age-dependent decrease of C16.

Nine out of 11 newborns with C5DC increase in renal insufficiency were boys; only two were girls. All patients with congenital renal insufficiency presenting with an increase of C5DC in newborn screening were boys.

Table 2 Clinical symptoms of infants with renal insufficiency presenting with an increase of glutarylcarnitine (C5DC) in extended newborn screening

Patient ID	Sex	Gestational age (weeks)	Birth weight (g)	C5DC in NBS (μmol/L)	Renal affection	
1	M	36	2,950	0.28	Congenital	Tubular dysgenesia (due to sartane intake during third trimenon of pregnancy)
2	M	41	2,770	0.19	Congenital	Renal hypoplasia/dysplasia
3	M	36	2,790	0.18	Congenital	Renal hypoplasia/dysplasia, died at age 3 weeks of life
4	M	37	3,030	0.13	Congenital	Polycystic kidney disease
5	M	35	2,400	0.16	Congenital	Hydronephrosis/ posterior urethral valves
6	M	23	622	0.10[a]	Acquired	Acute renal failure/ prematurity
7	M	26	570	0.12	Acquired	Acute renal failure/ prematurity
8	M	37	2,430	0.12	Acquired	Acute renal failure/ neonatal asphyxia
9	F	38	3,190	0.09[a]	Acquired	Acute renal failure/ neonatal asphyxia
10	M	42	3,550	0.10[a]	Acquired	Acute renal failure/ neonatal asphyxia
11	F	40	3,120	0.14	Acquired	Acute renal failure/ neonatal asphyxia

[a] In newborn screening (NBS), C5DC was at the upper cutoff level, but at least three C5DC/acylcarnitine ratios were elevated

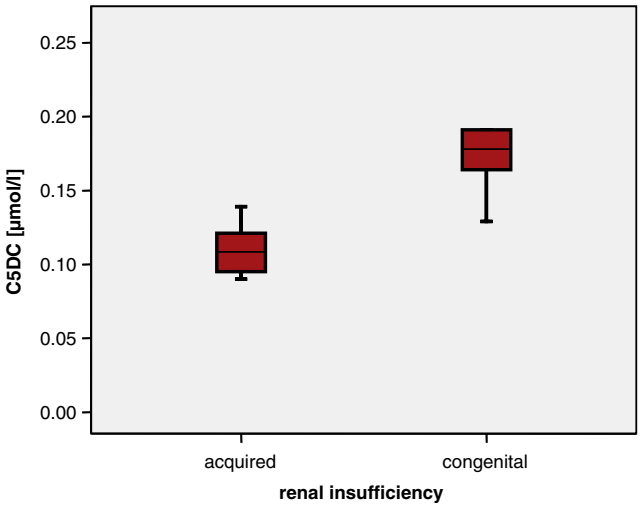

Fig. 1 Concentrations of glutarylcarnitine (C5DC) in newborn screening measured in children with acquired or congenital renal insufficiency. C5DC concentrations were significantly higher in children with congenital renal insufficiency than in those with acquired renal insufficiency (p=0.011)

Discussion

Pitfalls of newborn screening for GA I have been described occasionally. Mainly, children with a low excreting phenotype of GA I and low C5DC levels may be missed by newborn screening (Gallagher et al. 2005; Smith et al. 2001; Wilcken et al. 2003). An increase of C5DC not related to GA I has been demonstrated only in patients with MCADD due to pseudoglutarylcarnitinemia (Napolitano et al. 2004) and in patients with MADD. Furthermore, secondary glutarylcarnitinemia may be caused by maternal glutaric aciduria (Garcia et al. 2008). An isolated secondary urinary increase of glutaric acid, not related to GA I, was shown to be mediated by gut bacteria, resolving after antibiotic treatment (Wendel et al. 1995). Glutarylcarnitinemia associated with renal insufficiency has not yet been studied systematically.

Our data clearly indicate an association of glutarylcarnitinemia with renal disease. Children with congenital or acquired renal insufficiency were identified during newborn screening by an increased C5DC mimicking GA I. Pathogenesis of renal insufficiency seems to be important, as infants with congenital renal insufficiency showed significantly higher concentrations of C5DC and its ratios than infants with acquired renal insufficiency. There was a clear correlation between renal damage, expressed by an increase of creatinine and urea, and concentrations of C5DC. Obviously, glutarylcarnitinemia may be more extensive in children with renal insufficiency who are heterozygous for GA I.

Any elevation of C5DC should immediately prompt follow-up (Lindner et al. 2006). These data show that if C5DC elevation caused by GA I, MADD, or pseudoglutarylcarnitinemia due to MCADD is excluded, secondary glutarylcarnitinemia due to renal insufficiency has to be considered. In contrast to previously published data, our cutoff for C5DC seems quite low, but it was set at the 99.9th percentile to achieve 100% sensitivity (Lindner et al. 2006). Furthermore, in patients with milder forms of GA I, we measured C5DC levels as low as 0.13 µmol/L. The same concentrations of C5DC were measured in patients with renal insufficiency. The combination of C5DC with the secondary variables C5DC/(C8 + C10), C5DC/C16, and C5DC/C0 has been reported to have the highest specificity and sensitivity for diagnosing GA I in neonatal screening (Lindner et al. 2006). Our data reveal that these analytes may also be increased in neonates with renal insufficiency. The combination of C5DC with the variables C5DC/(C8 + C10), C5DC/C0, and C5DC/C8 showed the highest correlation with renal affection, but in contrast to children with GA1, C5DC/C16 was not highly correlated with renal insufficiency. This parameter may help to differentiate between glutarylcarnitinemia due to GA I and glutarylcarnitinemia due to renal insufficiency. However, any child with a positive newborn screening indicating GA needs further confirmation analysis, at least by determination of organic acids in urine. If glutarylcarnitinemia persists, enzymatic and/or genetic analysis should be performed as well.

Recently, active renal transporter systems for glutaric acid and glutaric acid derivates have been described (Mühlhausen et al. 2008; Smith et al. 2005; Yodoya et al. 2006). These transporters are important for the directed

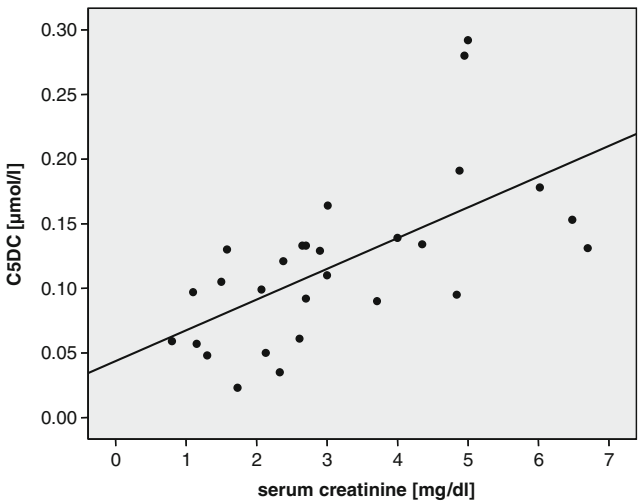

Fig. 2 Correlation of glutarylcarnitine (C5DC) and creatinine. Concentrations of C5DC in dried blood spots and creatinine in serum correlated significantly (p=0.001; r^2=0.393). Data shown in this figure present data for the examined infants from the time of first newborn screening until the 28th day of life. Per infant, a maximum of three values is included

uptake and excretion of the cytotoxic metabolite glutaric acid and its derivates in renal proximal tubular cells: sodium-dependent dicarboxylate cotransporter 3 (NaC3) and organic anion transporter 1 (OAT1), mediating the uptake of glutaric acid and its derivates from plasma at the basolateral site; and OAT4, mediating secretion of glutaric acid and its derivates into urine at the apical site. Renal damage in neonates might lead to disturbances in these renal transporter systems, resulting in a defect in the uptake or secretion of glutaric acid and its metabolites, causing an increase of plasma C5DC. Alternatively, neonatal renal insufficiency might result in decreased excretion of C5DC and thus in an increase of plasma C5DC.

Acquired renal disease was transient in all affected newborns, whereas congenital malformations were partly associated with persistent renal insufficiency. In one child, renal insufficiency was due to maternal treatment during third trimester of pregnancy with sartane, an angiotensin II type 1 receptor blocker. Angiotensin II receptor antagonists may have a well known teratogenic effect, resulting in oligohydramnions, fetal growth retardation, pulmonary hypoplasia, limb contractures, skull hypoplasia, and renal impairment, including tubular dysgenesia (Alwan et al. 2005; Bos-Thompson et al. 2005). Therefore, the application of sartane in the last two trimesters should be strictly avoided.

Conclusion

In newborns with an isolated increase of C5DC, renal insufficiency may be considered as a differential diagnosis to GA I. The pathomechanism may be explained by a decreased excretion of C5DC and thus in an increase of plasma C5DC.

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