Cerebral Organic Acidurias

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Summary

A group of organic acidurias, including Canavan disease (*N*-acetylaspartic aciduria), glutaric aciduria type I, L-2-HYDROXYLGUTARIC aciduria and D-2-hydroxyglutaric aciduria types I and II, are characterised by a predominantly or even exclusively neurological presentation and have therefore been termed 'cerebral'. Frequent neurological symptoms are motor and/or mental retardation or regression, extrapyramidal movement disorders and epilepsy. These symptoms are the result of acute and/or chronic pathological changes in various brain regions including grey matter (cortex, basal ganglia, cerebellum) and white matter (periventricular and subcortical). Unlike 'classic' organic acidurias (e.g. propionic and methylmalonic aciduria), acute metabolic decompensations with hyperammonemia, metabolic acidosis and elevated concentrations of lactate and ketone bodies are uncommon for cerebral organic acidurias. Biochemically, these diseases are characterised by accumulation of characteristic organic acids, mostly dicarboxylic acids, in body fluids. At high concentrations some of these may become neurotoxic. Since the blood–brain barrier has a low transport capacity for dicarboxylic acids, cerebral accumulation of dicarboxylic acids is facilitated. Impairment of brain energy metabolism is suggested to play a central role in the pathophysiology of this disease group. Metabolic treatment initiated in neonatally diagnosed patients with glutaric aciduria type I has significantly improved the neurological outcome, whereas current treatment strategies for the other cerebral organic acidurias are ineffective.

8.1 Introduction

Canavan disease (Van Bogaert-Bertrand disease, *N*-acetylaspartic aciduria) is caused by an autosomal recessively inherited deficiency of aspartoacylase (aminoacylase 2) which is exclusively expressed in oligodendrocytes. Most patients have the infantile form which generally manifests at 2–4 months of age with head lag, muscular hypotonia and

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macrocephaly, progressing to marked developmental delay, seizures, optic nerve atrophy, progressive spasticity and opisthotonic posturing (Matalon et al. 1995). Death usually occurs in a few years. However, the initial symptoms may already start at or shortly after birth (neonatal form) or after the age of 5 years (juvenile form). Cranial MRI studies show diffuse or exclusively subcortical involvement of the white matter due to spongiform myelinopathy and involvement of thalamus and globus pallidus. Diagnosis can be made by finding elevated *N*-acetylaspartate concentrations in urine using GC/MS analysis of organic acids. A decreased aspartoacylase activity in cultured skin fibroblasts and/or the identification of two disease-causing mutations in the *ASPA* gene localised on 17p13.3 confirms the diagnosis. *N*-acetylaspartate is formed in neurons and hydrolysed to L-aspartate and acetate by oligodendrocytes. No effective treatment exists for Canavan disease. Lithium citrate decreases brain *N*-acetylaspartate concentrations (Assadi et al. 2010) and glyceryl triacetate treatment supplies the brain with acetate (Segel et al. 2011). Although this treatment is considered as safe, there is still no proof for its therapeutic efficacy. Adenoviral transfer of the *ASPA* gene to the brain has been initiated but no follow-up data have been published (Leone et al. 2000).

Glutaric aciduria type I (glutaric acidemia type I) is caused by an autosomal recessively inherited deficiency of glutaryl-CoA dehydrogenase, an FAD-dependent mitochondrial matrix enzyme. This enzyme is involved in the catabolic pathways of lysine, hydroxylysine and tryptophan, catalysing the oxidative decarboxylation of glutaryl-CoA to crotonyl-CoA. Transient muscular hypotonia and macrocephaly is often found in newborns. At this age, cranial MRI of affected individuals reveals temporal hypoplasia, dilated external CSF spaces, subependymal pseudocysts, myelination delay and an immature gyral pattern which all may improve or even resolve in early treated children (Harting et al. 2009). In the time interval between 3 and 36 (−72) months, however, most untreated patients develop a complex movement disorder best described as generalised dystonia superimposed on axial hypotonia (Gitiaux et al. 2008). These symptoms are the consequence of bilateral striatal injury which may occur acutely during acute encephalopathic crises precipitated by catabolism or insidiously without preceding crises (Kölker et al. 2006). Aside from striatal injury, MRI may show additional frontal atrophy and subdural haemorrhage. A few adolescent and adult patients with a late-onset form have been reported presenting with headaches, vertigo, reduced fine motor skills and white matter abnormalities (Harting et al. 2009). Patients can be identified in the first week of life by newborn screening using glutarylcarnitine as diagnostic parameter. Glutarate and 3-hydroxyglutarate concentrations are increased in urine and other body fluids but may be (intermittently) normal in patients with a low-excreter phenotype. Therefore, a normal organic acid analysis result does not unequivocally exclude the diagnosis. Suspected diagnosis is confirmed by significantly decreased glutaryl-CoA dehydrogenase activity in leukocytes or fibroblasts and/or the identification of two disease-causing mutations in the *GCDH* gene localised on 19p13.2. Glutarate and 3-hydroxyglutarate concentrations are 100–1,000-fold higher in the brain than in plasma which is caused by the very low efflux transport of dicarboxylic acids across brain capillary endothelial cells (Sauer et al. 2006). At high concentrations, accumulating dicarboxylic acids may become neurotoxic inhibiting energy metabolism (2-oxoglutarate dehydrogenase, dicarboxylate shuttle between astrocytes and neurons) and activating *N-methyl-d-aspartate* receptors. The development of prognostic relevant striatal injury can be prevented in the majority of children if the diagnosis is made and metabolic treatment is started neonatally (Heringer et al. 2010). Metabolic treatment according to a recently revised guideline includes a low lysine diet, carnitine supplementation and emergency treatment to counteract catabolism (Kölker et al. 2011). Patients with a high- and low-excreter phenotype have the same risk of developing striatal injury and thus receive the same treatment (Kölker et al. 2006).

L-2-hydroxyglutaric aciduria (L2HGA) is an autosomal recessive inborn error of metabolism, caused by mutations in the *L2HG dehydrogenase* (*L2HGDH*) gene. The *L2HGDH* gene product, i.e. L2HGDH, is an FAD-dependent membrane-bound enzyme responsible for the conversion of L-2-hydroxyglutarate (L2HG) into 2-ketoglutaric acid (2KG). The current opinion is that nonspecific mitochondrial formation of L2HG out of 2KG by L-malic dehydrogenase is the sole source of L2HG and that L2HGDH is an enzyme for metabolite repair (Van Schaftingen et al. 2009). L2HGA has an insidious onset starting in childhood with developmental delay, macrocephaly, epilepsy and cerebellar ataxia as clinical signs. In a minority of patients, the diagnosis is established in adulthood, but retrospective evaluation of the clinical course reveals an earlier subtle onset (Steenweg et al. 2010). MRI reveals disease-specific alterations characterised by predominantly subcortical cerebral white matter abnormalities and abnormalities of the dentate nucleus, globus pallidus, putamen and caudate nucleus (Steenweg et al. 2009). Metabolic investigations will reveal increased 2HG in the urinary organic acid screening, and subsequent chiral differentiation shows the increased excretion of exclusively

L2HG. Apart from the massive increase of L2HG in all body fluids, a modest increase of CSF lysine is observed, while plasma lysine levels may be normal. Since the massive increase of L2HG is the major biochemical finding, pathology is likely to be explained by the pathologic levels of L2HG; however, lowered (peripheral) 2KG levels might also attribute to the disease. Currently, there is no established treatment protocol for L2HGA apart from two anecdotic reports mentioning positive effects of treatment with riboflavin and/or FAD.

D-2-hydroxyglutaric aciduria (D2HGA) type I is one of the two subtypes of D2HGA and has an autosomal recessive pattern of inheritance. The disease is caused by mutations in the *D2HG dehydrogenase* (*D2HGDH*) gene, resulting in a deficiency of D-2-hydroxyglutarate (D2HG) dehydrogenase (Struys et al. 2005). This FAD-dependent mitochondrial enzyme converts D2HG, most likely formed by the action of hydroxyacid:oxoacid transhydrogenase (HOT), into 2KG. Although several hypothetical metabolic pathways for D2HG have been proposed, there is strong evidence that D2HG is directly and exclusively formed out of 2KG (Struys et al. 2004). The disease displays a strong clinical heterogeneity from severely affected individuals to asymptomatic individuals. However, frequently reported clinical findings are developmental delay, hypotonia and epilepsy. Usually, patients are first recognised by an increase of 2HG in the urinary organic acid screening. In contrast with L2HGA, these elevations can be modest. The increase of D2HG in all body fluids is the sole biochemical alteration in this disease, and the pathophysiology of the disease is likely to be explained by this. Currently, there is no treatment. However, it can be hypothesised that in individual cases, riboflavin supplementation might be beneficial.

D-2-hydroxyglutaric aciduria (D2HGA) type II is the second form of D2HGA and is caused by a gain-of-function mutation in the *isocitrate dehydrogenase 2* (*IDH2*) gene (Kranendijk et al. 2010). Heterozygous mutations in IDH2 result in the formation of a neomorph enzyme which is able to efficiently convert 2KG into D2HG. This is in contrast with the normal action of IDH2, i.e. the conversion of isocitrate into 2KG. D2HGA type II has an autosomal dominant trait, and in the vast majority of patients, the mutation arose de novo. The degree of D2HG accumulation in D2HGA type II is higher than in type I, despite properly functioning D2HG dehydrogenase. Patients affected with D2HGA type II suffer from developmental delay, hypotonia and epilepsy, and their clinical presentation is generally more severe than that of patients with D2HGA type I. Cardiomyopathy is frequently observed in D2HGA type II and absent in type I. The unique underlying mechanism of D2HGA type II opens perspectives to specifically inhibit the neomorph enzyme.

A most recent review covering L2HGA and both types of D2HGA was published by Kranendijk et al. (2012).

8.2 Nomenclature

No.	Disorder	Alternative name	Abbreviation	Gene symbol	Chromosomal localisation	Affected protein	OMIM no.	Subtype
8.1	Canavan disease	Van Bogaert-Bertrand disease	CD	*ASPA*	17p13.3	Aspartoacylase (aminoacylase 2)	271900	All forms
8.2	Glutaric aciduria type I	Glutaric acidemia type I	GA-I	*GCDH*	19p13.2	Glutaryl-CoA dehydrogenase	231670	All forms
8.3	L-2-hydroxyglutaric aciduria	L-2-hydroxyglutarate dehydrogenase deficiency	L2HGA	*L2HGDH*	14q21.3	L-2-hydroxyglutarate dehydrogenase	236792	All forms
8.4	D-2-hydroxyglutaric aciduria type I	D-2-hydroxyglutarate dehydrogenase deficiency	D2HGA type I	*D2HGDH*	2q37.3	D-2-hydroxyglutarate dehydrogenase	600721	All forms
8.5	D-2-hydroxyglutaric aciduria type II	Isocitrate dehydrogenase defect	D2HGA type II	*IDH2*	15q26.1	Isocitrate dehydrogenase 2	613657	All forms

8.3 Metabolic Pathways

Canavan Disease

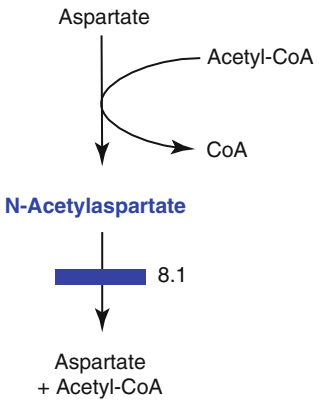

Fig. 8.1 Metabolic pathway of Canavan disease. *N*-acetylaspartate is produced in neurons from L-aspartate and acetyl-CoA and is transported to oligodendrocytes where it is hydrolysed to L-aspartate and acetyl-CoA by aspartate aminoacylase II. Inherited deficiency of this enzyme results in accumulation of *N*-acetylaspartate in patients with Canavan disease

Glutaric Aciduria Type I

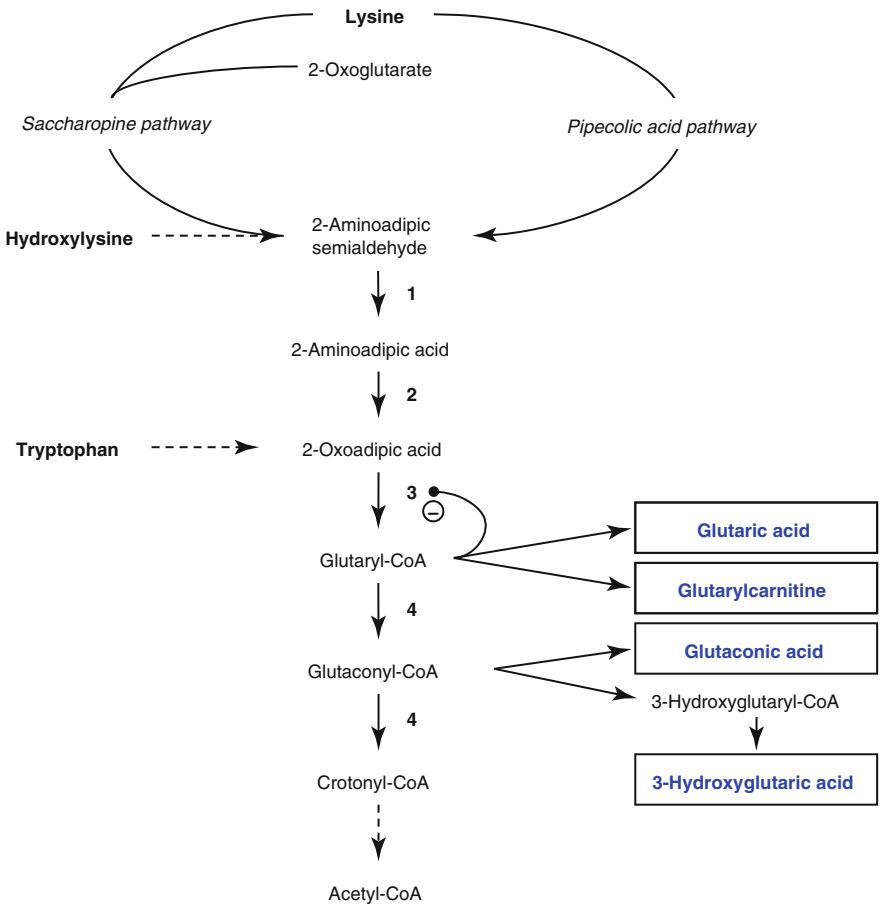

Fig. 8.2 Metabolic pathway of glutaric aciduria type I. Glutaryl-CoA is formed within the catabolic pathways of lysine, tryptophan and hydroxylysine. The quantitatively major precursor is lysine. Deficient activity of glutaryl-CoA dehydrogenase (4) results in variable accumulation of glutaric, 3-hydroxyglutaric and glutaconic acid as well as glutarylcarnitine, which are important for the diagnosis and can be determined in body fluids. Elevated glutaryl-CoA – similarly to homologous succinyl-CoA – results in feedback inhibition of the TCA cycle enzyme 2-oxoglutarate dehydrogenase complex (3). *1* 2-aminoadipic semialdehyde dehydrogenase (enzyme is deficient in pyridoxine-dependent epilepsy), *2* 2-aminoadipate aminotransferase, *3* 2-oxoglutarate dehydrogenase-like complex (enzyme complex contains three subunits: DHTKD1 is deficient in 2-aminoadipic/2-oxoadipic aciduria; the E2 subunit is deficient in 2-oxoglutarate dehydrogenase complex deficiency; E3 subunit (lipoamide dehydrogenase), which is shared with pyruvate dehydrogenase and branched-chain oxoacid dehydrogenase complexes, is deficient in E3 deficiency, biochemically resembling maple syrup urine disease, 2-oxoglutarate dehydrogenase complex deficiency and pyruvate dehydrogenase complex deficiency, *4* glutaryl-CoA dehydrogenase

L-2-Hydroxyglutaric Aciduria

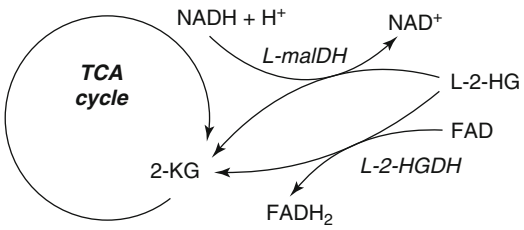

Fig. 8.3 Metabolic pathway of L-2-hydroxyglutaric aciduria. L-2-hydroxyglutaric acid is formed by a nonspecific conversion of mitochondrial 2KG into L-2-hydroxyglutaric acid by the action of NADH-dependent L-malic acid dehydrogenase. L2HG dehydrogenase corrects for this metabolic imperfection by reconverting L2HG into 2KG

D-2-Hydroxyglutaric Aciduria Type I

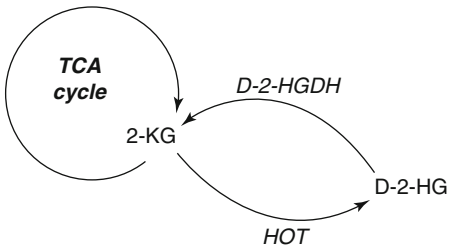

Fig. 8.4 Metabolic pathway of D-2-hydroxyglutaric aciduria type I. D-2-hydroxyglutaric acid is formed out of mitochondrial 2KG by the action of hydroxy acid-oxoacid transhydrogenase (*HOT*). D2HG is reconverted into 2KG by D2HG dehydrogenase. This pathway is believed to play a role in GABA/GHB homeostasis

D-2-Hydroxyglutaric Aciduria Type II

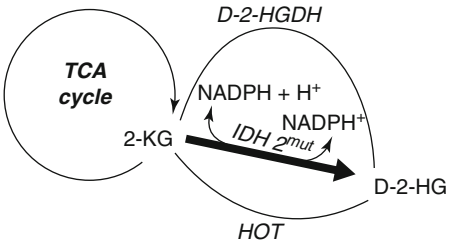

Fig. 8.5 Metabolic pathway of D-2-hydroxyglutaric aciduria type II. As a consequence of a heterozygous mutation in IDH2, the neomorph IDH2 enzyme produces vast amounts of D2HG, which exceed the capacity of D2HG dehydrogenase, an enzyme with a low Km, and as a net result, D2HG accumulates. The neomorph IDH2 enzyme consumes both 2KG and NADPH, which might lead to their shortages

8.4 Signs and Symptoms

Table 8.1 Canavan disease

System	Symptom	Neonatal	Infancy	Childhood	Adolescence	Adulthood
CNS	Decerebrate posture			±	±	±
	Dysarthria	±	±	±	±	±
	Epilepsy	±	±	±	±	±
	Extrapyramidal movement disorder	±	±	±	±	±
	Loss of very early milestones		+	+	+	+
	Mental retardation	+	+	+	+	+
	Motor retardation	+	+	+	+	+
	Muscular hypotonia	±	+	+	±	±
	Opisthotonous	±	±	±	±	±
	Spasticity		±	+	+	+
Ear	Deafness	±	±	±	±	±
Eye	Blindness		+	+	+	+
	Nystagmus	±	±	±	±	±
	Optic atrophy		+	+	+	+
Musculoskeletal	**Macrocephaly**	+	+	+	+	+
Special laboratory	MRI: bilateral subcortical leukodystrophy + involvement of globus pallidus	±	+	+	+	+
	N-acetylaspartic acid (CSF, P)		↑	↑	↑	↑
	N-acetylaspartic acid (U)		↑	↑	↑	↑

Most patients follow the infantile form which mostly manifests at 2–4 months of age with head lag, muscular hypotonia and macrocephaly, progressing to marked developmental delay, seizures, optic nerve atrophy, progressive spasticity and opisthotonic posturing (Matalon et al. 1995)

Table 8.2 Glutaric aciduria type I

System	Symptom	Neonatal	Infancy	Childhood	Adolescence	Adulthood
CNS	Abasia		±	±	±	±
	Astasia			±	±	±
	Ataxia			±	±	±
	Chorea		±	±	±	±
	Dysarthria		±	±	±	±
	Dystonia		±	±	±	±
	Encephalopathic crisis, acute		±	±		
	Headache				±	±
	Hypokinesia		±	±	±	±
	Hypotonia, axial	±	±	±		
	Spasticity			±	±	±
	Swallowing difficulties		±	±	±	±
	Vertigo				±	±
Digestive	Feeding difficulties		±	±	±	±
	Vomiting		±	±	±	±
Musculoskeletal	**Macrocephaly**	±	+	±	±	±
Respiratory	Pneumonia		±	±	±	±
Routine laboratory	ASAT/ALAT (P)		(↑)	(↑)	(↑)	(↑)
	Creatine kinase (P)		(↑)	(↑)	(↑)	(↑)
Special laboratory	3-Hydroxyglutaric acid (U)	n-↑	n-↑	n-↑	n-↑	n-↑
	C5DC glutarylcarnitine (P, B)	n-↑	n-↑	n-↑	n-↑	n-↑
	Glutaconic acid (U)	n-↑	n-↑	n-↑	n-↑	n-↑
	Glutaric acid (U)	n-↑	n-↑	n-↑	n-↑	n-↑
	MRI: periventricular white matter and other extrastriatal MR abnormalities		±	±	+	+
	MRI: striatal atrophy		±	±	±	±
	MRI: temporal hypoplasia, dilated external CSF spaces	+	+	±	±	±

Table 8.3 L-2-hydroxyglutaric aciduria

System	Symptom	Neonatal	Infancy	Childhood	Adolescence	Adulthood
CNS	**Ataxia**		±	+	+	+
	Choreoathetosis			±	±	±
	Dysarthria		±	±	±	±
	Dystonia			±	±	±
	Hypotonia	±	±	±	±	±
	Mental retardation		±	+	+	+
	Seizures		±	±	±	±
	Spasticity			±	±	±
	Tremor			±	±	±
Musculoskeletal	**Macrocephaly**		±	±	±	±
Routine laboratory	Ammonia (B)	n-↑				
	Lactate (P)	n-↑				
	Protein (CSF)		↑	↑		
Special laboratory	L-2-hydroxyglutaric acid (U, P, CSF)	↑	↑	↑	↑	↑
	Lysine (P, CSF)	n	n-↑	n-↑	n-↑	n-↑
	MRI/CT: white matter abnormalities	±	+	+	+	+
	MRI: globus pallidus and dentate nudeus lesions	±	+	+	+	+

Table 8.4 D-2-hydroxyglutaric aciduria type I

System	Symptom	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Cardiovascular	Cardiomyopathy	n	n	n	n	n
CNS	**Developmental delay**	+	+	+	+	+
	Epilepsy	±	±	±	±	±
	Hypotonia	+	+	+	±	±
Special laboratory	D2HG (U, P, CSF)	↑	↑	↑	↑	↑

Table 8.5 D-2-hydroxyglutaric aciduria type II

System	Symptom	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Cardiovascular	Cardiomyopathy	±	±	±	±	±
CNS	**Developmental delay**	+	+	+	+	+
	Epilepsy	+	+	+	+	+
	Hypotonia	+	+	+	±	±
Special laboratory	D2HG (U, P, CSF)	↑	↑	↑	↑	↑

8.5 Reference Values

N-acetylaspartic acid (NAA)

	NAA (U)	NAA (P)	NAA (CSF)
Age	mmol/mol creatinine	µmol/L	µmol/L
	6–36	0.17–0.84	0.25–2.8

Gas chromatography/mass spectrometry (preferably with stable isotope dilution assay)

Glutaric acid (GA)

	GA (U)	GA (P)	GA (CSF)
Age	mmol/mol creatinine	µmol/L	µmol/L
	< 10	0.5–2.9	0.18–0.63

Gas chromatography/mass spectrometry (preferably with stable isotope dilution assay)

3-Hydroxyglutaric acid (3-OH-GA)

	3-OH-GA (U)	3-OH-GA (P)	3-OH-GA (CSF)
Age	mmol/mol creatinine	µmol/L	µmol/L
	< 8	0.2–1.36	< 0.2

Gas chromatography/mass spectrometry (preferably with stable isotope dilution assay)

Glutarylcarnitine (C5DC)

	C5DC (DBS)	C5DC (P)	C5DC (U)
Age	mmol/mol creatinine	µmol/L	µmol/L
	< cut-off[a]	< cut-off[a]	< cut-off[a]

Tandem mass spectrometry
[a]Cut-off needs to be set by each lab

L-2-hydroxyglutaric acid (L2HG)

	L2HG (U)	L2HG (P)	L2HG (CSF)
Age	mmol/mol creatinine	µmol/L	µmol/L
	1–19	0.5–1	0.3–2.3

Separation and individual quantification of the D- and L-isomer of 2HG by gas chromatography–mass spectrometry or liquid chromatography-tandem mass spectrometry

D-2-hydroxyglutaric acid (D2HG)

	D2HG (U)	D2HG (P)	D2HG (CSF)
Age	mmol/mol creatinine	µmol/L	µmol/L
	3–17	0.3–0.9	0.07–0.3

Separation and individual quantification of the D- and L-isomer of 2HG by gas chromatography–mass spectrometry or liquid chromatography-tandem mass spectrometry

8.6 Pathological Values

Disease 8.1 (CD): *N*-Acetylaspartic Acid (NAA)

N-acetylaspartic acid (NAA)

	NAA (U)	NAA (P)	NAA (CSF)
Age	mmol/mol creatinine	µmol/L	µmol/L
	60–10,000	Up to 10	

Gas chromatography/mass spectrometry (preferably with stable isotope dilution assay)

Disease 8.2 (GA-I): Glutaric Acid (GA), 3-Hydroxyglutaric Acid (3-OH-GA), Glutarylcarnitine (C5DC)

Glutaric acid (GA)

	GA (U)	GA (P)	GA (CSF)
Age	mmol/mol creatinine	µmol/L	µmol/L
	N-10,000	N-200	N-40

Gas chromatography/mass spectrometry (preferably with stable isotope dilution assay)

3-Hydroxyglutaric acid (3-OH-GA)

	3-OH-GA (U)	3-OH-GA (P)	3-OH-GA (CSF)
Age	mmol/mol creatinine	µmol/L	µmol/L
	N-500	N-30	N-5

Gas chromatography/mass spectrometry (preferably with stable isotope dilution assay)

Glutarylcarnitine (C5DC)

	C5DC (DBS)	C5DC (P)	C5DC (U)
Age	mmol/mol creatinine	µmol/L	µmol/L
	> cut-off[a]	> cut-off[a]	> cut-off[a]

Tandem mass spectrometry
[a]Cut-off needs to be set by each lab

Disease 8.3 (L2HGA)

	L2HG (U)	L2HG (P)	L2HG (CSF)
Age	mmol/mol creatinine	µmol/L	µmol/L
	226–4,299	7–84	23–474

Separation and individual quantification of the D- and L-isomer of 2HG by gas chromatography–mass spectrometry or liquid chromatography-tandem mass spectrometry

Disease 8.4 (D2HGA type I)

	D2HG (U)	D2HG (P)	D2HG (CSF)
Age	mmol/mol creatinine	µmol/L	µmol/L
	103–2,414	26–123	6–18

Separation and individual quantification of the D- and L-isomer of 2HG by gas chromatography–mass spectrometry or liquid chromatography-tandem mass spectrometry

Disease 8.5 (D2HGA type II)

	D2HG (U)	D2HG (P)	D2HG (CSF)
Age	mmol/mol creatinine	µmol/L	µmol/L
	448–11,305	99–757	30–172

Separation and individual quantification of the D- and L-isomer of 2HG by gas chromatography–mass spectrometry or liquid chromatography-tandem mass spectrometry

8.7 Diagnostic Flow Chart(s)

Disease 8.1 (CD)

If neurological symptoms occur and/or neuroradiological abnormalities are found (→**8.5**, Signs and symptoms) which are characteristic for Canavan disease, the diagnostic process should be initiated by analysis of *N*-acetylaspartate (NAA) in urine. If increased NAA concentrations are found, the diagnosis can be confirmed by significantly decreased aspartoacylase activity in cultured skin fibroblasts and/or the identification of two disease-causing mutations in the *ASPA* gene.

Disease 8.2 (GA-I)

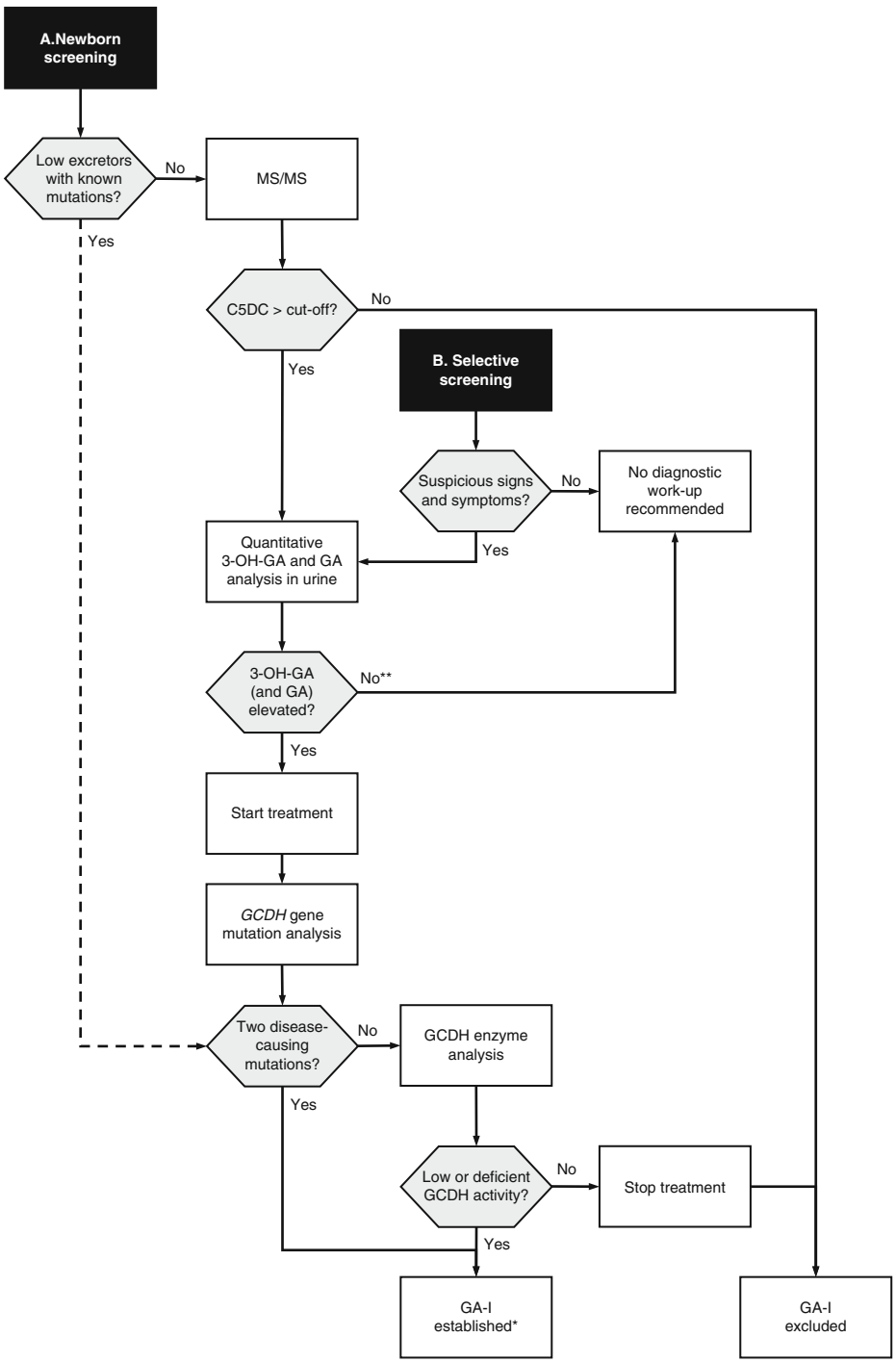

Fig. 8.6 Diagnostic flow chart for glutaric aciduria type I. *A. Newborn screening* for glutaric aciduria type I (GA-I) is performed using tandem mass spectrometry (MS/MS). In low-excreter cohorts with known mutations, *GCDH* gene mutation analysis should be considered as alternative method (*dotted line*). Note that in these patients, treatment should be started after the identification of two disease-causing mutations (*). *B. Selective screening* should be started if diagnosis of GA-I is suspected or a positive family history is known. Note that a few patients with a low-excreting phenotype may show (intermittently) normal urinary excretion of 3-hydroxyglutaric acid (*3-OH-GA*) and glutaric acid (*GA*). If an individual shows normal 3-OH-GA (and GA) concentrations in urine but presents with highly suspicious signs and symptoms for GA-I, it should be decided individually whether diagnostic work-up is continued (**)

Diseases 8.3–8.5 (L2HGA, D2HGA type I and D2HGA type II)

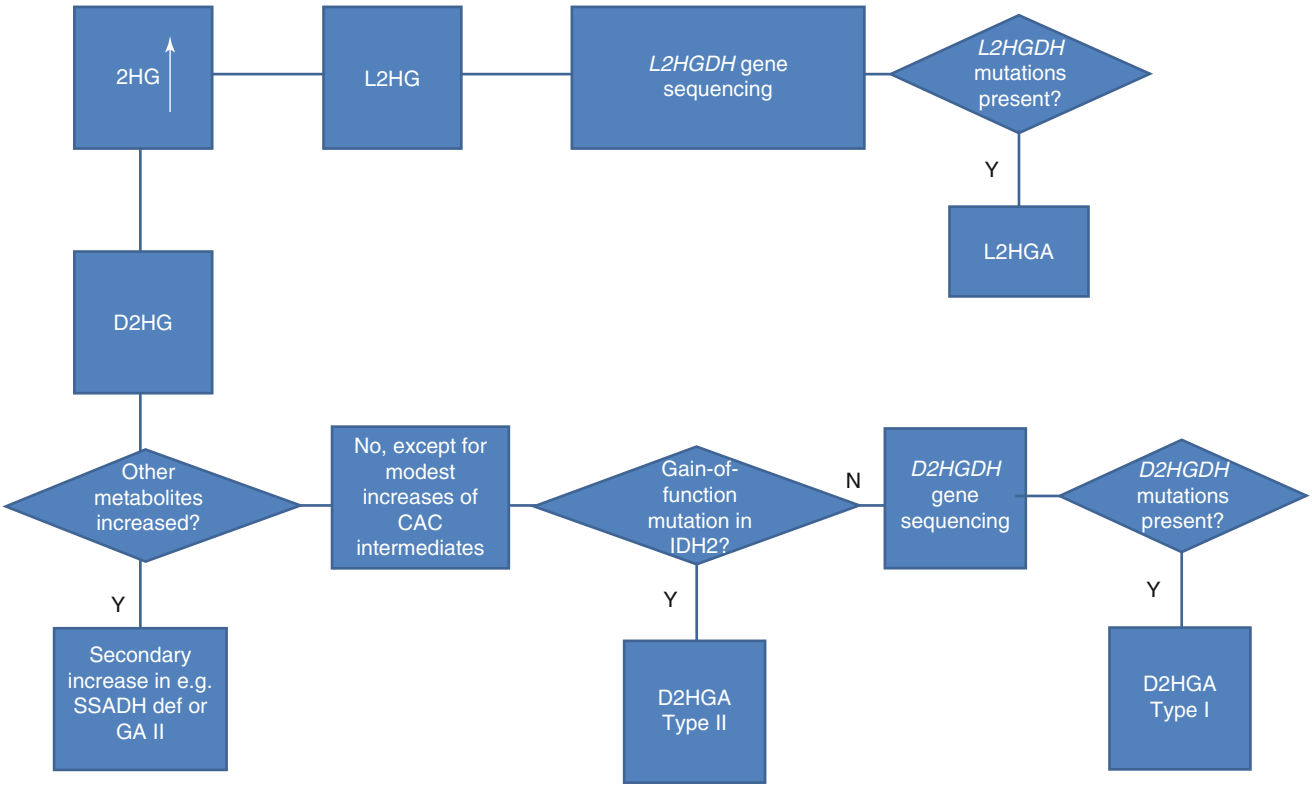

Fig. 8.7 Flowchart for the diseases **8.3**, **8.4** and **8.5**. The observed increase in 2HG in the urinary organic acid screening has to be pursued by an enantiomeric separation and individual quantification of both D2HG and L2HG. In case of an isolated increase of L2HG, subsequent molecular investigation of the *L2HGDH* gene usually reveals mutations confirming the biochemical diagnosis. In case of an isolated increase of D2HG, the diagnostic algorithm is more complicated. D2HG (whereas L2HG is within the reference range) can be secondary increased in SSADH deficiency and GA-I. If these options are excluded, molecular investigation on the two known heterozygous gain-of-function mutations will either exclude or confirm mutated IDH2 as the cause of the D2HG increase. If IDH2 molecular studies are negative, the *D2HGDH* gene should be investigated for the presence of mutations

8.8 Specimen Collection

Disease no.	Symbol	Test	Preconditions	Material	Handling	Pitfalls
8.1	CD	Organic acids (NAA)	None	Urine	Keep frozen (−20 °C)	Compound has poor recovery in organic solvent extraction
8.2	GA-I	Quantitative amino acids	3.5–4 h postprandially, no dietary changes prior to the test	Plasma	Keep frozen (−20 °C)	
		Trytophan	3.5–4 h postprandially, no dietary changes prior to the test	Plasma	Keep frozen (−20 °C)	Losses due to inappropriate deproteinisation
		Organic acids (3-OH-GA, GA)	None	Urine	Keep frozen (−20 °C)	Reliable identification of 3-OH-GA may require the use of a quantitative GC/MS method; differential diagnoses of elevated GA and 3-OH-GA include GA-II and GA-III, SCHAD deficiency and ketosis
		Carnitine status	None, also informs on adherence to carnitine supplementation	Plasma serum	Keep frozen (−20 °C)	
		Acylcarnitine profile (C5DC)	None	Dried blood spots Plasma	Plasma, keep frozen (−20 °C)	C5DC may be also elevated in GA-II, renal insufficiency, MCAD deficiency (pseudoglutarylcarnitinemia)
		Enzyme activity (GCDH)	None	Fibroblasts Leukocytes from heparinised blood	RT Keep frozen (−20 °C)	
8.3	L2HGA	Organic acids	None	Urine	Keep frozen (−20 °C)	For specific quantification of L2HG enantiomeric separation, hyphenated to mass spectrometry is required
	L2HGA	L2HG dehydrogenase activity	Isolation of cells according to specific protocol	Fibroblasts, lymphoblasts, lymphocytes	RT, pellets should be frozen	
8.4	D2HGA type I	Organic acids	None	Urine	Keep frozen (−20 °C)	For specific quantification of D2HG enantiomeric separation, hyphenated to mass spectrometry is required
	D2HGA type I	D2HG dehydrogenase activity	Isolation of cells according to specific protocol	Fibroblasts, lymphoblasts	RT, pellets should be frozen	
8.5	D2HGA type II	Organic acids	None	Urine	Keep frozen (−20 °C)	For specific quantification of D2HG enantiomeric separation, hyphenated to mass spectrometry is required D2HG also accumulates GA-I and SSADH
	D2HGA type II	IDH2 gain-of-function assay	Isolation of cells according to specific protocol	Lymphoblasts	RT, pellets should be frozen	

3-OH-GA 3-hydroxyglutarate, *C5DC* glutarylcarnitine, *GA* glutarate, *GA-II* glutaric aciduria type II, *GA-III* glutaric aciduria type III, *GCDH* glutaryl-CoA dehydrogenase, *GC/MS* gas chromatography/mass spectrometry, *MCAD* medium-chain acyl-CoA dehydrogenase, *NAA* *N*-acetylaspartate, *SCHAD* short-chain 3-hydroxyacyl-CoA, *SSADH* succinic semialdehyde dehydrogenase deficiency

Prenatal diagnosis table for all disorders (if applicable) and sample requirements

Disease no.	Symbol	Material	Timing trimester	Pitfalls
8.1	CD	Chorionic villi (accomplished) Amniotic fluid, amniocytes (accomplished)	I II	Assay of aspartoacylase in amniocytes is not reliable. A combination of mutation analysis together with the exact quantitation of *N*-acetylaspartate in the amniotic fluid is recommended
8.2	GA-I	Chorionic villi Amniocytes, amniotic fluid	I II	
8.3	L2HGA	Chorionic villi Amniocytes, amniotic fluid	I II	
8.4	D2HGA type I	Chorionic villi Amniocytes, amniotic fluid	I II	
8.5	D2HGA type II	Chorionic villi Amniocytes, amniotic fluid	I II	Disease is usually caused by a heterozygous de novo mutation. Somatic and germline mosaicism in the parents cannot be excluded; thus, the recurrence risk for the parents is not zero. Therefore, prenatal diagnosis should always be offered

In case of DNA-based prenatal diagnosis, maternal contamination should be excluded by VNTR marker analysis
Trimester one: only mutation analysis
Trimester two: a combination of metabolite and DNA investigations is recommended

DNA testing table for all disorders (if applicable) and sample requirements

Disease no.	Symbol	Tissue	Methodology
8.1	CD	Lymphocytes, fibroblasts	Sequencing
8.2	GA-I	Lymphocytes, fibroblasts	Sequencing
8.3	L2HGA	Blood, lymphocytes, fibroblasts, lymphoblasts	Sequencing
8.4	D2HGA type I	Blood, lymphocytes, fibroblasts, lymphoblasts	Sequencing
8.5	D2HGA type II	Blood, lymphocytes, fibroblasts, lymphoblasts	Sequencing, targeted mutation analysis

8.9 Treatment Summary

Effective metabolic treatment has only been described for glutaric aciduria type I (low lysine diet, carnitine supplementation, emergency treatment). Riboflavin should be considered as a treatment option for patients with L-2-hydroxyglutaric aciduria aiming to activate residual enzyme activity. Treatment of patients' Canavan disease with lithium citrate, lowering brain *N*-acetylaspartate concentrations, and glycerol triacetate, supplying the brain with acetate, is considered as safe; however, it is yet unknown whether it helps to improve the outcome. Metabolic treatment for D-2-HYDROXYGLUTARIC aciduria type I and II has not yet been described.

Although effective treatment is only known for some cerebral organic acidurias, symptomatic and supportive treatment is important. This includes adequate supply with nutrient, minerals and micronutrients, physiotherapy, occupational therapy and pharmacotherapy of epilepsy and extrapyramidal movement disorder among others. The therapeutic concept should be implemented after the assessment of individual needs and, subsequently, monitored and evaluated by an interdisciplinary team of specialists.

Emergency Treatment Table for All Disorders of Your Chapter (If Applicable) **and Medication Requirements** (A. Including Box After the Table, with Pitfalls and Important Information)

Diseases 8.1 and 8.3–8.5

No emergency treatment is available.

Disease 8.2 (GA-I)

Emergency treatment is thought to be the most effective component of current treatment strategies to prevent acute striatal injury during infectious disease and for other causes of catabolism in glutaric aciduria type I (Heringer et al. 2010). It must be initiated before the onset of severe neurological signs, which already indicate the manifestation of neuronal damage. Therefore, during episodes that are likely to induce catabolism (e.g. infectious disease) emergency treatment should start without delay. Treatment should consist of frequent high carbohydrates feeds and increased carnitine supplementation, followed by high-dose intravenous glucose and carnitine (Kölker et al. 2011). All patients with glutaric aciduria type I should be supplied with an emergency card. This concept should be strictly followed for the first 6 years of life. After this age emergency treatment is individually adjusted.

Emergency treatment at home

A. Oral carbohydrates[a]	**Maltodextran**			
Age (years)	%	kcal/100 mL	KJ/100 mL	Volume (mL) per day orally
Up to 0.5	10	40	167	min. 150/kg
0.5–1	12	48	202	120/kg
1–2	15	60	250	100/kg
2–6	20	80	334	1,200–1,500
B. Protein intake				
Natural protein	Stop for max 24 (−48) h, then reintroduce and increase stepwise until the amount of maintenance treatment is reached within 48 (−72) h			
AAM	If tolerated, AAM should be administered according to maintenance therapy			
C. Pharmacotherapy				
L-carnitine	Double oral carnitine supplementations			

According to Kölker et al. 2011
[a]Solutions should be administered every 2 h day and night. Patients should be reassessed every 2 h. AAM, lysine-free, tryptophan-reduced amino acids mixtures

Emergency treatment in hospital (according to Kölker et al. 2011)

A. Intravenous infusions		
Glucose	Age (years)	Glucose (g/kg per day IV)
	0–1	(12–) 15
	1–3	(10–) 12
	3–6	(8–) 10
Insulin	If persistent hyperglycemia >150 mg/dL (>8 mmol/L) and/or glucosuria occurs, start with 0.05 IE insulin/kg per h IV and adjust the infusion rate according to serum glucose	
B. Protein intake		
Natural protein	Stop for max 24 (−48) h, then reintroduce and increase stepwise until the amount of maintenance treatment is reached within 48 (−72) h	
AAM	If tolerated, AAM should be administered orally or by nasogastric tube according to maintenance therapy	
C. Pharmacotherapy		
L-carnitine	100 (−200) mg/kg per day IV	

AAM, lysine-free, tryptophan-reduced amino acid mixtures

Standard Treatment Table for All Disorders of Your Chapter (if applicable) **and Medication Requirements** (A. Including Box After the Table, with Pitfalls and Important Information)

Diseases 8.1, 8.3, 8.4 and 8.5
No specific treatment is available.
Disease 8.2 (GA-I)

Disorder no.	Symbol	Age	Medication/diet	Dosage	Doses/day (n)
8.2	GA-I	<6 years	Carnitine	(50–) 100 mg/kg per day	3
		>6 years	Carnitine	(30–) 50 mg/kg per day	3
			Riboflavin[a]	100 mg	2
			Treatment of extrapyramidal movement disorders[b]		
			Antiepileptic treatment[c]		
			Diet (see below)		

[a]Riboflavin responsiveness seems to be a very rare occasion in glutaric aciduria type I and no standard protocol exists to test it. There is no proven clinical benefit of riboflavin supplementation (Kölker et al. 2006)
[b]The complex movement disorder of symptomatic patients with glutaric aciduria type I is often difficult to treat. Baclofen and benzodiazepames as monotherapy or in combination should be used as first-line drug treatment for focal and generalised dystonia. Intrathecal baclofen should be considered as additional therapy for generalised dystonia and spasticity. Trihexyphenidyl should be considered as second-line treatment for dystonia, particularly in adolescents and adults. Botulinum toxin A injections should be considered as additional therapy for severe focal dystonia (according to Kölker et al. 2011)
[c]Epilepsy is not frequently found in glutaric aciduria type I. Dystonic and epileptic movements, however, may be confused. Indication, prescription and monitoring of antiepileptic treatment should be performed by a child neurologist or neurologist. Valproate should *not* be used due to the theoretical risk of mitochondrial dysfunction and secondary carnitine depletion

Dietary treatment (low lysine diet, according to Kölker et al. 2011)

Treatment	Dosage	Age				
		0–6 months	7–12 months	1–3 years	4–6 years	>6 years
Lysine (from natural protein)[a]	mg/kg per day	100	90	80–60	60–50	Avoid excessive intake of natural protein; use natural protein with a low lysine content according to 'safe levels'
Protein from amino acid mixtures[b]	g/kg per day	1.3–0.8	1.0–0.8	0.8	0.8	
Energy	kcal/kg per day	115–80	95–80	95–80	90–80	
Micronutrients[c]	%	≥100	≥100	≥100	≥100	≥100

[a]The lysine/protein ratio (i.e., 2–9 mg lysine/100 mg protein) varies in natural food and thus natural protein intake in children on a low lysine diet is dependent on the natural protein source
[b]Lysine-free, tryptophan-reduced amino acid mixtures should be preferably supplemented with minerals and micronutrients. Safe intakes of essential amino acids are provided from natural protein and lysine-free, tryptophan-reduced amino acid supplements
[c]According to dietary recommendations

Dangers/Pitfalls

1. Dietary treatment needs to be adapted to the individual needs, in particular in dystonic patients. Overtreatment by protein restriction may result in malnutrition with essential nutrients.
2. Dysphagia is a frequent problem in dystonic patients. Tube feeding (via nasogastric tube or percutaneous gastrostomy) should be considered if oral food intake is inadequate.

Experimental Treatment for All Disorders of Your Chapter (If Applicable) **and Medication Requirements** (A. Including Box After the Table, with Pitfalls and Important Information)

Disease no.	Symbol	Alternative therapies/experimental trials
8.1	CD	Lithium citrate Glycerol triacetate Gene therapy
8.2	GA-I	Arginine or homoarginine supplementation has yet only been studied in GCDH-deficient mice, an animal model for GA-I, and in a small number of patients (arginine)
8.3	L2HGA	Riboflavin supplementation
8.4	D2HGA type I	On the basis that D2HG dehydrogenase is an FAD-dependent enzyme, riboflavin supplementation is a therapeutic option
8.5	D2HGA type II	Development of specific inhibitor of the IDH2 mutant enzyme

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