Glutaric aciduria type I: Enzymatic and neuroradiologic investigations of two kindreds

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Two kindreds with glutaric aciduria type I were investigated. Of 20 family members who underwent neurologic examination and organic acid analysis of urine, 18 had glutaryl-coenzyme A dehydrogenase (GDH) activity determined in cultured skin fibroblasts and 12 had computed tomographic brain scans. Six homozygotes were identified who had undetectable GDH activity and identical biochemical profiles (consisting of glutaric and 3-hydroxyglutaric aciduria, reduced serum carnitine concentrations, and frontotemporal atrophy). Serial computed tomographic brain scans of one homozygous infant demonstrated the sequential postnatal development of this atrophy during 3 years before the development of clinical manifestations. In three of the six homozyotes, including the father in one kindred, there were no clinical manifestations of glutaric aciduria type I. These findings raise questions about the value of prenatal diagnosis in predicting clinical manifestations in homozygous newborn infants. (J PEDIATR 1989;114:983-9)

Glutaric aciduria type I is an inherited metabolic disease caused by deficiency of glutaryl–coenzyme A dehydrogenase activity. This mitochondrial enzyme oxidizes and decarboxylates glutaryl-coenzyme A, an intermediate step in the degradation of lysine and tryptophan.[1,2] The clinical manifestation is generally acute, with a metabolic crisis or an encephalitis-like illness in infancy. Symptoms are primarily extrapyramidal, dominated by dystonia and chorcoathetosis. Seizures, developmental delay, and mental retardation have also been reported.[1,3-13] The diagnosis of GA-I is determined by organic acid analysis of urine demonstrating elevated excretion of glutaric and 3-hydroxyglutaric acids, and is confirmed by measuring the activity of GDH in cultured skin fibroblasts or leukocytes.[1,14] Pedigree studies[2,7,15] have identified individuals who are homozygotes or heterozygotes for the enzymatic defect by determining GDH activity in leukocytes in three generations of two kindreds. No asymptomatic individuals proved homozygous for the enzymatic defect have been reported.

We have recently reported two sibling pairs from two different kindreds.[16] All four children had undetectable GDH activity in cultured fibroblasts and frontotemporal atrophy on computed tomographic scans, yet one child from each family was asymptomatic. These findings led us to undertake extensive investigation of the two kindreds.

See related article, p. 1004.

CT	Computed tomography
GA-I	Glutaric aciduria type I
GABA	γ-Aminobutyric acid
GAD	Glutamic acid decarboxylase
GDH	Glutaryl–coenzyme A dehydrogenase

METHODS

All 20 family members underwent neurologic examination by one of us (R.A. or R.S.), who had no knowledge of the results of other investigations. Urinary organic acids,

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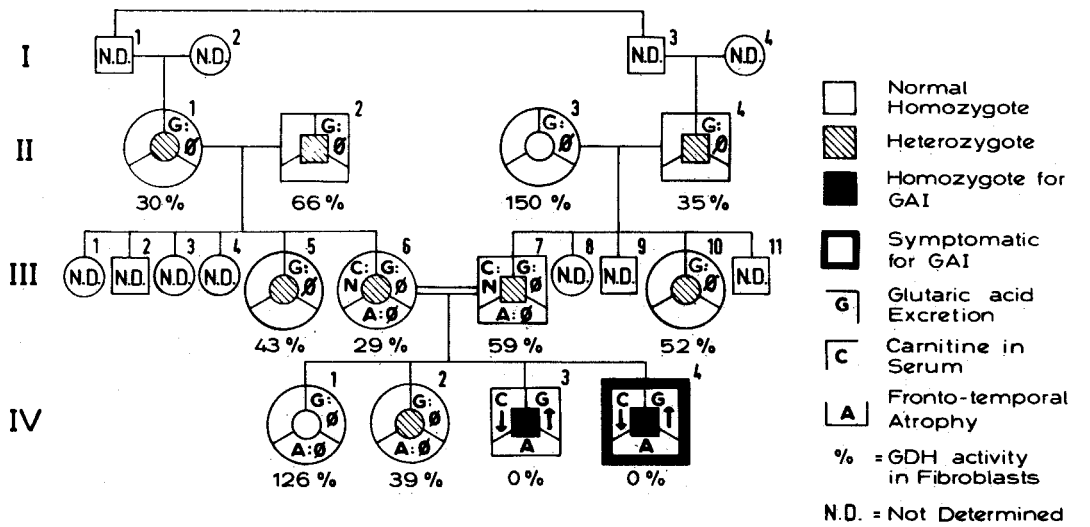

Fig. 1. Pedigree of three generations in kindred 1, including biochemical profiles, clinical symptoms, brain CT scan findings, and GDH activity in fibroblasts.

Table. Biochemical features of homozygotes with undetectable GDH activity in GA-1

	Age (y)	Urinary glutaric acid excretion (mmol/L per mmol/L creatinine)	Serum carnitine (free/total) ratio (nmol/ml)	Urinary carnitine (free/total) ratio (mmol/L per mmol/L creatinine)
Kindred I				
IV-3	11	1.42-2.34	1.7/4.2	1.7/11.4
IV-4	1¼	1.26-3.96	9.6/20.0	2.86/3.2
Kindred II				
I-3	37	0.78-0.81	1.5/4.1	0.34/13.8
II-14	6	1.81	2.9/5.3	
II-15	3	1.28-4.02	2.7/6.1	
II-16	½*	1.09-5.92	19.7/34.1	1.5/117
	2†		3.0/5.7	
Control		0-0.43	>21/30	>50% Free

*Received carnitine supplementation.
†After carnitine supplementation was stopped.

as their trimethyl silyl derivatives,[17] were analyzed by high-performance liquid chromatography–mass spectrometry for all 20 individuals. Glutaric acid was quantitated by selected ion monitoring analysis.

Assay of GDH activity in cultured fibroblasts was performed in 18 individuals as previously described.[14] Two siblings of the propositus in kindred 2 (II-3) and (II-8) refused skin biopsy and CT scan.

A CT scan of the brain was performed for 6 people in each kindred: the propositi, both their parents, and three asymptomatic siblings.

Serum L-carnitine levels were determined by radioenzymatic assay[18] in all homozygotes and in three heterozygotes for the enzymatic defect. Plasma levels of very long chain fatty acids were determined in both propositi and one asymptomatic homozygous sibling in each kindred by a method modified from Aubourg et al.[19]

RESULTS

Kindred 1. Twelve people were investigated in three generations of this kindred (Fig. 1), which is of Kurdish-Jewish ancestry (tending to consanguineous marriages). The parents of the propositus are second cousins.

Results of the neurologic examination were normal in 11 family members. Only one individual, the propositus (IV-4), who has been severely debilitated by dystonic encephalopathy since 4 months of age, had any neurologic signs.[16] Urinary organic acid analysis identified two individuals,

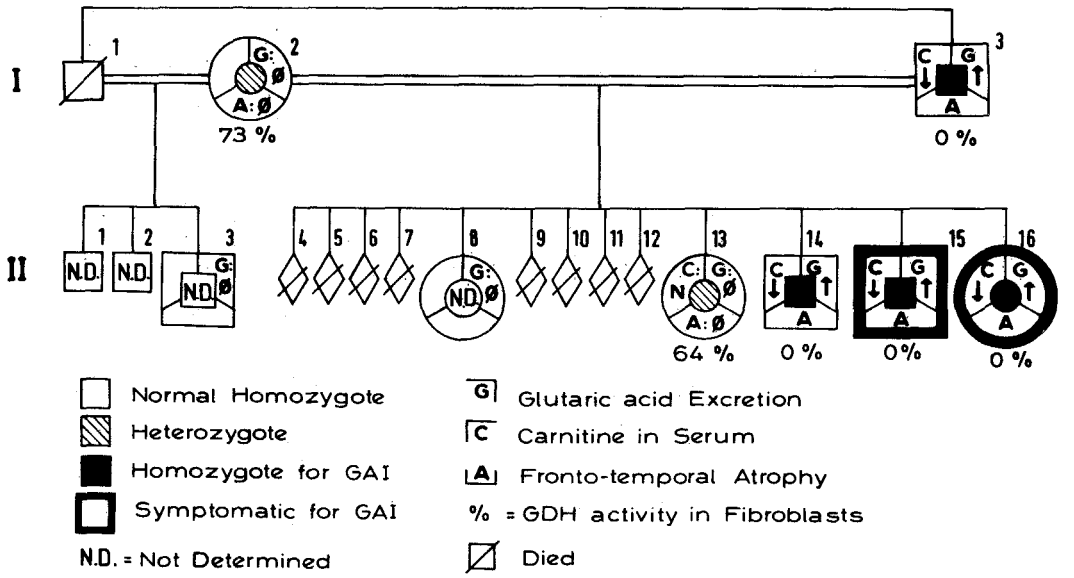

Fig. 2. Pedigree of two generations in kindred 2, including biochemical profiles, clinical symptoms, CT findings, and GDH activity in fibroblasts.

the propositus (IV-4) and his 11-year-old asymptomatic brother (IV-3), who excreted excessive amounts of glutaric and 3-hydroxyglutaric acids in urine (Table).

Activity of GDH in cultured fibroblasts was not detectable in the two individuals with excessive glutaric and 3-hydroxyglutaric acid excretion; hence they were identified as homozygous for the enzymatic defect. Eight individuals, including the obligate heterozygous parents, had enzyme activities ranging from 29% to 66% of normal values and were considered heterozygotes. The paternal grandmother (II-3) and one sister of the propositus (IV-1) were normal, with enzyme activities of 150% and 126%, respectively. Serum carnitine levels were normal in both parents and reduced in both homozygotes with GDH deficiency (Table). The muscle carnitine level was reduced in the asymptomatic sibling (IV-3) to 0.24 nmol/mg wet tissue (normal is >1.50 nmol/mg wet tissue). Plasma levels of very long chain fatty acids (C26/C22 ratio) were normal in both siblings with GDH deficiency.

The CT scans of the parents (III-6 and III-7) and their two daughters (IV-1 and IV-2) were normal. The CT scan of the propositus (IV-4) and his brother (IV-3), who are homozygous for GDH deficiency, demonstrated frontotemporal atrophy. This atrophy was more severe in the propositus, who also had hypodense areas in white matter, enlarged ventricles with loss of caudate nucleus, and diffuse cortical atrophy.

Kindred 2. Kindred 2 (Fig. 2) is of Moslem ancestry, with a history of consanguineous marriages. The parents of the propositus are first cousins. There were 13 children, of whom 8 died in infancy. Both parents and the five surviving children are included in this study. The mother was married previously to the brother of her present husband. They had three children, all of whom are healthy adults. One of these children (II-3) is also included in this study. Eight persons were examined; seven were neurologically normal. The propositus (II-15), a 4-year-old boy, has had severe dystonia since experiencing an encephalitis-like episode at 4 months of age.[16] A younger sister (II-16), 2½ years old at the time of study, was mildly delayed in psychomotor development but had no neurologic signs.[16] She had been diagnosed as having GA-I at 1 month of age and had received L-carnitine supplementation until 18 months of age, when it was discontinued by the parents. The parents had failed to comply with long-term dietary manipulation and riboflavin therapy during her infancy. A short-term low-lysine diet was followed by a reduction in urinary glutaric acid excretion, whereas L-carnitine supplementation enhanced this excretion. At the age of 3 years, during a mild intercurrent illness, she suddenly developed a Reye syndrome–like condition that left her severely debilitated with dystonic quadriparesis.

Urinary organic acid analysis was performed in eight people. Four individuals excreted abnormal amounts of glutaric and 3-hydroxyglutaric acids in urine: the propositus (II-15), his father (I-3), the 2½-year-old sister (II-16), and the 6-year-old brother (II-14) (Table).

All four individuals with glutaric aciduria were homozy-

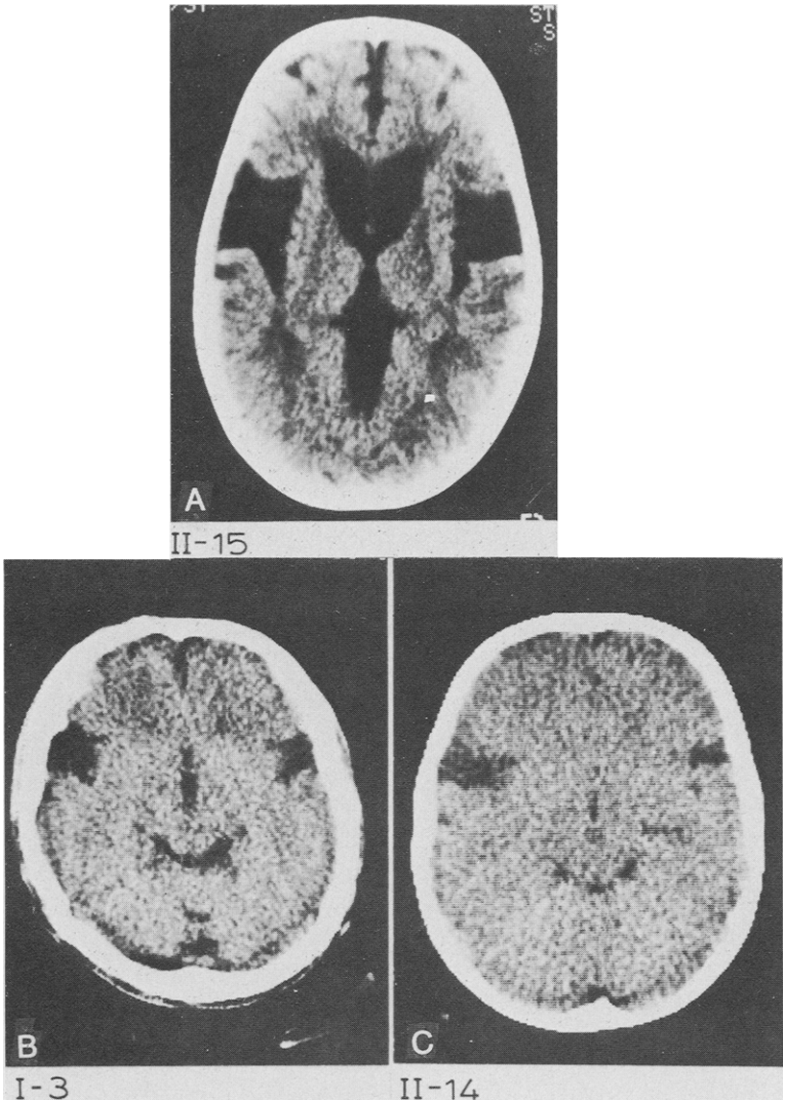

Fig. 3. Brain CT scan of homozygotes in kindred 2. **A,** Scan of propositus (II-15) when 15 months old. Note greatly enlarged insular cisterns, frontotemporal atrophy, white matter hypodensities, and enlarged ventricles. **B,** Scan of father (I-3) of propositus. Note frontotemporal atrophy with enlarged insular cisterns. **C,** Scan of 6-year-old asymptomatic sibling (II-14). Note localized frontotemporal atrophy.

gotes for the enzymatic defect, with undetectable activity in cultured skin fibroblasts. The mother (I-2), an obligate heterozygote, had GDH activity of 73%; the 8-year-old daughter (II-13) had activity of 64% and was a presumed heterozygote. The 14-year-old daughter (II-8) and the adult son from the first marriage (II-3) refused skin biopsy. Serum carnitine levels were reduced in all four homozygotes for GDH deficiency (Table) but were normal in the heterozygous sister (II-13). The C26/C22 ratio in plasma was normal for both the propositus and his asymptomatic homozygous brother (II-14).

A CT scan of brain in all four individuals with GDH deficiency showed localized frontotemporal atrophy (Fig. 3). In the propositus and his sister (II-16), this atrophy was much more severe and was accompanied by ventricular enlargement, white matter hypodensities, and diffuse cortical atrophy. Serial CT scans (Fig. 4) of patient II-16 demonstrated white matter hypodensity without cortical atrophy at 6 weeks of age and the development of frontotemporal atrophy by 1 year with normalization of the white matter; at 18 months mild subdural effusion was seen, which resolved spontaneously by 2 years of age. At 2½ years of age, when she was still asymptomatic, the CT scan disclosed diffuse cortical atrophy, pronounced fronto-

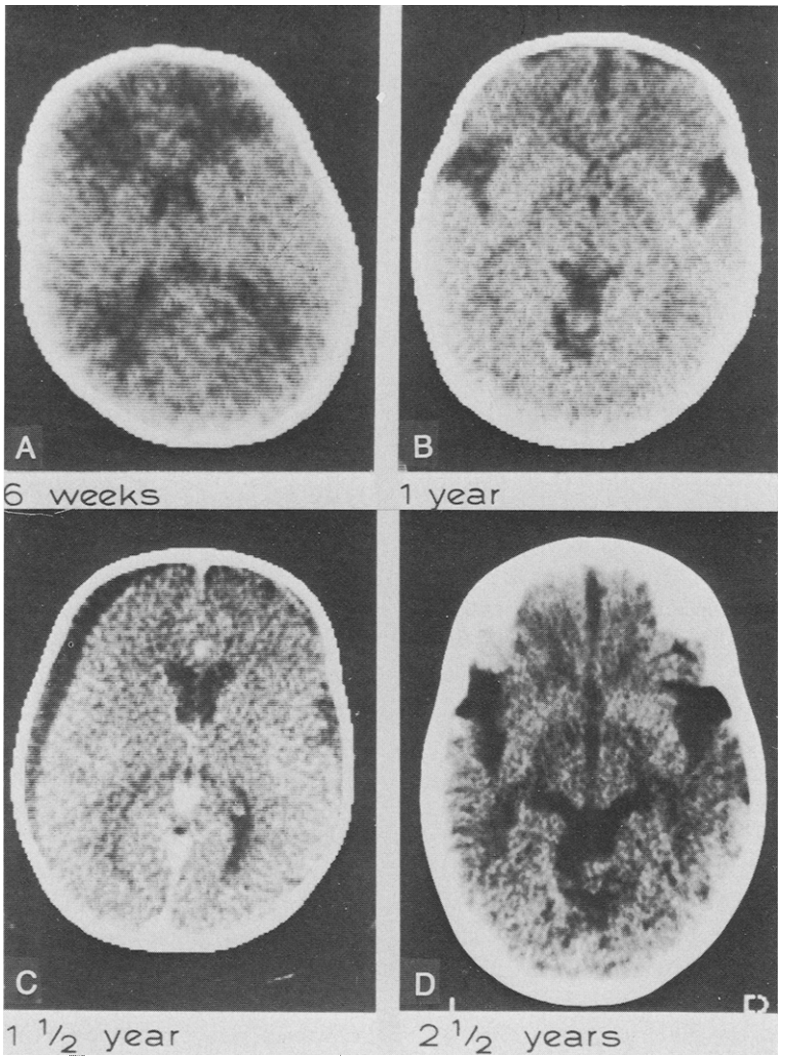

Fig. 4. Serial brain CT scans of patient II-16 in kindred 2. **A,** At 6 weeks of age white matter hypodensities are excessive for neonatal period, with no cortical atrophy. **B,** At 1 year of age there is frontotemporal atrophy with enlarging insular cisterns and normalization of white matter. **C,** At 1½ years of age there is subdural effusion over left convexity. Localized atrophy is still seen on right. **D,** At 2½ years of age, subdural effusion has resolved spontaneously, leaving localized frontotemporal atrophy much more pronounced. White matter hypodensities and diffuse atrophy are also seen.

temporal atrophy, and white matter hypodensities. The mother and 8-year-old sister have normal CT scans.

DISCUSSION

These investigations enabled us to classify the family members into three distinct groups on the basis of enzyme activity: 6 homozygotes with undetectable GDH activity, 10 heterozygotes with intermediate enzyme activity ranging from 29% to 73%, and the normal subjects with 150% to 126% activity. Enzymatic activity of those individuals presumed to be heterozygotes was in the range of that of the obligate heterozygotes. We found no overlap between the enzyme activities in heterozygotes and in homozygotes for GDH deficiency. The six homozygotes had identical biochemical profiles consisting of glutaric and 3-hydroxyglutaric aciduria and reduced serum carnitine levels, as well as the consistent CT finding of frontotemporal atrophy. Three of these six homozygotes had none of the clinical manifestations heretofore associated with GA-I. We know of no previous evidence that undetectable GDH activity can be compatible with the asymptomatic state well into adulthood.

Frontotemporal atrophy was found in all six homozygotes with GDH deficiency, regardless of clinical condition. None of the asymptomatic individuals ever had encephalitis or metabolic crises that could be implicated as

a cause of this atrophy. The localized atrophy and enlarging insular cisterns are not congenital but develop postnatally during infancy, as shown by serial CT scans of one patient. It does not appear to progress beyond early childhood in asymptomatic homozygous individuals; in the adult it is no more extensive than in the child. In the symptomatic patient, the frontotemporal atrophy is much more remarkable. Yamaguchi et al.[13] have recently reported a similar pattern of localized atrophy in a 7-month-old infant with GA-I and have suggested that this radiologic finding is specific for this disorder. Although it is a rare condition, we have observed it in other severe infantile encephalopathies of unknown cause in which GA-I has been excluded and it has been reported in West syndrome.[20] In both propositi and in patient II-16 of kindred 2, it was accompanied by diffuse cortical atrophy, greatly enlarged mesencephalic cisterns, white matter hypodensities, and symmetric enlargement of frontal horns. Despite the generalized biochemical disturbance and the extensive brain abnormalities demonstrated on CT scan, the motor manifestations are confined to those of the basal ganglia (dystonia and choreoathetosis).

The pathophysiologic processes causing the dystonic encephalopathy of GA-I are probably associated with neurotransmitter metabolism, specifically that of γ-aminobutyric acid and glutamate. Biochemical analysis of the brains of two children with GA-I disclosed elevated levels of glutaric acid in the frontal cortex and basal ganglia, very low glutamic acid decarboxylase activity in the substantia nigra, and extremely low concentrations of GABA in the caudate and putamen.[8,21] The demonstration by Stokke et al.[22] that GAD is inhibited by glutaric acid in vitro is relevant to the above findings. The markedly reduced GABA concentrations in both caudate and putamen suggest antemortem deficiency of GAD activity in the areas in which glutaric acid was increased. The clinical manifestations of dystonia correlate well with putaminal dysfunction.[23]

It has been suggested that abnormally increased glutamatergic neurotransmission is a factor in the pathogenesis of dystonic cerebral palsy[24] and neurodegenerative diseases of the basal ganglia such as Huntington chorea.[25] The structural similarity of glutamic and glutaric acids might promote abnormal glutamatergic synaptic dynamics in two ways. Glutaric acid might interfere with the reuptake of glutamate, resulting in its accumulation in the synapse, or it could activate glutamatergic binding sites, resulting in enhanced glutamatergic neurotransmission.[26]

Patient II-16 from kindred 2 was diagnosed during the neonatal period and treated before symptoms developed with L-carnitine supplementation during the first 18 months of life. Unlike those of her asymptomatic brother and father, serial CT scans of her brain disclosed progressive changes in cortex and white matter resembling the changes seen in her symptomatic brother. However, she remained asymptomatic neurologically until developing a Reye syndrome–like disease at the age of 3 years. It is a matter of conjecture whether the administration of L-carnitine during infancy contributed to her neurologically asymptomatic state and/or delayed the development of the metabolic crisis. The combined administration of a low-lysine diet, riboflavin, and L-carnitine has been attempted in symptomatic individuals with very limited, if any, effect.[1,4,12,16,27] The severe neuroradiologic deterioration between the ages of 1½ and 2½ years in this girl developed after the discontinuation of L-carnitine supplementation. We suggest that the progressive deterioration shown on the CT scans be used as a marker to differentiate the presymptomatic from the truly asymptomatic state.

The major question raised by the results of our investigations concerns the mechanism by which the asymptomatic state can exist in the presence of an inborn error of metabolism that has hitherto been consistently associated with the development of neurologic manifestations in infancy or early childhood. Variability in nutritional intake, which could have resolved this question, has been excluded in these kindreds by interview. A peroxisomal oxidation of glutaryl-CoA has been proposed as an alternative to the mitochondrial route[28] and could be implicated as a detoxifying pathway. This oxidation would lead to the formation of glutaconic acid, which, in the absence of GDH, could not undergo decarboxylation. We believe that the accumulation of glutaconic acid, in itself a toxic substance, would not be compatible with the asymptomatic state. Moreover, it has been postulated that in patients with GA-I, in whom glutaryl-CoA accumulates, oxidation by peroxisomes would interfere with peroxisomal oxidation of very long chain fatty acids, resulting in raised plasma levels of these acids.[29] However, plasma levels of these acids were normal in both propositi and in one asymptomatic homozygous sibling in each family; thus this process cannot be implicated as a cause of the asymptomatic state. Interference with the metabolism of very long chain fatty acids might exist in patients with GA-I during acute illness, but this is unverified.

The fact that marked clinical variability among homozygous individuals with identical biochemical profiles occurs within the same kindred with consanguineous parents excludes GDH allelic heterogeneity and suggests the existence of unrelated, genetically polymorphic protective mechanisms. These mechanisms could be related to the vulnerability of glutamatergic networks in the basal ganglia or, alternatively, to GAD vulnerability to elevated glutaric acid concentrations.

Prenatal diagnosis of GA-I has been made by determination of glutaric acid concentration in amniotic fluid and GDH activity in cultured amniotic cells.[30] Exclusion of GA-I in the first trimester of pregnancy is also possible by determinating GDH activity in a sample of chorionic villi (unpublished data). The value of prenatal diagnosis in predicting the development of clinical manifestations could be questioned in view of the results of our investigation of two kindreds, in which undetectable GDH activity was not synonomous with the development of clinical disease. In such families, the development of the clinical manifestations may ultimately be determined by other, unrelated, genetically polymorphic mechanisms. In view of the considerable risk of individuals with GDH deficiency developing the severely debilitating symptoms of GA-I, genetic counseling and prenatal diagnosis should be offered to parents who have one affected child. In presymptomatic infants, L-carnitine supplementation might be effective in preventing or delaying clinical manifestations.

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