Am. J. Hum. Genet. 59:1012–1018, 1996

Glutaric Aciduria Type I in the Arab and Jewish Communities in Israel

Y. Anikster,[1] A. Shaag,[1] A. Joseph,[2] H. Mandel,[3] B. Ben-Zeev,[4] E. Christensen,[5] and O. N. Elpeleg[1]

[1]Metabolic Disease Unit and [2]Pediatric Neurology Unit[2], Shaare-Zedek Medical Center, Jerusalem; [3]Department of Pediatrics B, Rambam Medical Center, Haifa; [4]Pediatric Neurology Unit, Sheba Medical Center, Tel-Aviv; and [5]Department of Clinical Genetics, Rigshospitalet, Copenhagen

Summary

Mutation analysis was performed in eight families (16 patients) with glutaric aciduria type I (GA-I), which were all the families diagnosed in Israel in the years 1987–1994. Six families were of Moslem origin and two were non-Ashkenazi Jews. The entire coding region of the cDNA of the glutaryl-CoA dehydrogenase gene was sequenced in one patient of each family. Seven new mutations were identified in 15 of 16 mutated alleles, including six point mutations: T416I (4 alleles), G390R (1 allele), and S305L, A293T, L283P, and G101R (2 alleles each). In addition, a 1-bp deletion at position 1173 was identified in two alleles. These findings do not provide a molecular basis for the clinical variability in GA-I families. The occurrence of multiple novel mutations in a small geographic area may be explained by their recent onset in isolated communities with a high consanguinity rate.

Introduction

Glutaric aciduria type I (GA-I) is an inborn error of metabolism due to deficiency of the mitochondrial enzyme glutaryl-CoA dehydrogenase (GCD) (E.C. 1.3.99.7). GCD is a mitochondrial homotetramer that dehydrogenates glutaryl-CoA to crotonyl-CoA in the oxidation pathway of lysine, hydroxylysine, and tryptophan, in the mitochondria. The result of the enzymatic reaction is the reduction of the enzyme-bound flavin adenine diphosphate (FAD). Patients present during the first year of life with an acute encephalitis-like disease associated with an intercurrent infection. On recovery from the acute disease, multiple neurologic abnormalities including quadriplegia, choreoathetosis, and dystonic posturing, persist in most patients. Clinical variability is common, and some patients insidiously develop dystonia and choreoathetosis, yet others remain nearly asymptomatic (Amir et al. 1987, 1989; Haworth et al. 1991; Hoffmann et al. 1991; Morton et al. 1991; Kyllerman et al. 1994; Goodman and Frerman 1995).

The human gene for GCD has been localized to chromosome 19p13.2 (Greenberg et al. 1994). The gene is ~7 kb long and comprises 11 exons and 10 introns. The open reading frame of the cDNA consists of 1,314 nt and encodes a 438–amino acid precursor protein with a cleavable 44 residue sequence for mitochondrial import (Goodman et al. 1995). A point mutation at the +5 position of intron 1 of the GCD gene was identified in all the aboriginal patients from the Island Lake communities (Greenberg et al. 1995).

Subjects and Methods

Subjects

Eight families with GA-I were available for this study. All the patients and most of their siblings underwent a complete physical and neurological examination. Urinary organic acid analysis was performed for all available first-degree relatives. Family 1 was of Kurdish-Jewish origin, family 4 was of Iraqi-Jewish origin, and the other families were Moslem Arabs. Families 2, 5, and 6 were residents of villages in Hebron area, and families 7 and 8 were from villages in the lower Galilee. The clinical data of the families are presented in table 1. Family 1 and 2 were previously described by Amir et al. (1989), and family 8 was reported by Mandel et al. (1991). In all families, the parents were first- or second-degree cousins. The families were not aware of any relation to each other. The index case in each family presented before 2 years of age with an encephalitis-like disease, except for patient 7, who presented with subacute hemiparesis. The diagnosis of the index case was followed by detection of at least one more affected sibling in five of the families, bringing the total number of affected individuals in these families to 16. The clinical history of additional eight dead siblings in family 2 and

Received April 18, 1996; accepted for publication August 1, 1996.

Address for correspondence and reprints: Dr. Orly N. Elpeleg, Metabolic Disease Unit, Shaare-Zedek Medical Center, Jerusalem 91031, Israel. E-mail: elpeleg@cc.huji.ac.il

This article is dedicated to the memory of Prof. Naomi Amir.

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0002-9297/96/5905-0008$02.00

Table 1

Clinical Findings in the Index Cases and Their Affected Relatives

Patient	Sex of Index Case/ No. of Siblings[a]	Age at Onset of Neurological Abnormalities	Findings at Last Examination	Findings in Affected Siblings
1 (Amir et al. 1989)	M/3	4 mo—acute hypotonia, dystonia, and quadriparesis	Quadriplegic dystonia and anarthria, died at 8 years of age	Asymptomatic 20-year-old brother
2 (Amir et al. 1989)	M/12	6 mo—acute hypotonia, dystonia, and quadraplegia	6 years—severe dystonic quadriparesis and anarthria	Asymptomatic father and 10 year-old brother, 5-year-old symptomatic sister
3	M/6	8 mo—acute hypotonia, dystonia, and quadraplegia	15 mo—marked hypotonia and quadriplegia	Elder brother presented with acute quadriplegia at 6 mo and died at 12 years of age
4	F/2	18 mo—convulsions and coma	4 years—hypotonia, muscle weakness, and speech delay	Nine-year-old brother with motor dyspraxia and learning disabilities
5	M/3	10 mo—acute hypotonia, dystonia, and quadriplegia	3 years—dystonic posturing	Asymptomatic 6-year-old sister, 1-year-old sister with severe retardation and quadriplegia (lissencephaly and GA-I)
6	F/3	1 year—acute dystonia	3 years—normal psychomotor development	Seven-year-old sister with dystonic quadriparesis
7	F/4	10 mo—subacute hemiparesis	8 years—walks freely but suffers from muscle hypertonia and ataxia. Normal mental and social development	
8 (Mandel et al. 1991)	M/3	3 mo—suspected hydrocephalus, 6 mo—acute hypotonia and dystonia	8 years—learning disability, normal muscle tone	

[a] M = male; F = female.

one dead sibling in family 3 was suggestive of GA-I. The diagnosis of GA-I was established in all 16 subjects by the detection of excessive excretion of glutaric and 3-hydroxyglutaric acids in urine. The activity of GCD was undetectable in the fibroblasts of the index case in all families apart from family 3 and 5, whose fibroblasts were not available. Marked serum carnitine deficiency was found in all 16 patients. The course and outcome were variable among and within the families. Seven patients remained severely impaired with dystonic-dyskinetic syndrome accompanied in most by quadriparesis; five patients suffered from motor incoordination, moderate dyskinesia and attention-deficit disorder; and four were asymptomatic, including the father, individual I-1, of family 2 . The brain computed tomography (CT) scan of all affected individuals showed frontotemporal atrophy (brain CT scan was not performed in the patients of family 6); loss of caudate nuclei was found in patient II-4 of family 1, patients II-12 and II-13 of family 2, patient II-7 of family 3, and patients II-3 and II-4 of family 5; the CT scan of patient II-4 of family 5 also showed lissencephaly.

DNA Analysis

Genomic DNA was extracted, and cDNA was produced from fibroblasts of the father and the four children (II-1 to II-4) of family 1 (fig. 1), the parents and two children (II-11 and II-13) of family 2, the fresh blood of the parents and children (II-1, II-2, and II-4 to II-7) of family 3, the parents and all three children of family 4, the parents and all four children of family 5, the parents and children II-1 and II-4 of family 6, the parents and the index case II-2 in family 7, and the parents and the index case II-1 in family 8. Genomic DNA was extracted by standard methods. Total RNA was isolated using Tri reagent (Molecular Research Center) according to the manufacturer's instructions. First-strand cDNA was generated from total RNA by use of Moloney Murine Leukemia Virus reverse transcriptase (Promega) with the antisense oligonucleotide primer 4R (Sambrook et al. 1989). A 1,562-bp fragment of cDNA was amplified from the reverse-transcribed product by use of the oligonucleotide primers 1F and 4R (Greenberg et al. 1995). Four nested fragments were PCR-amplified from the 1,562-bp fragment by use of the four sets of

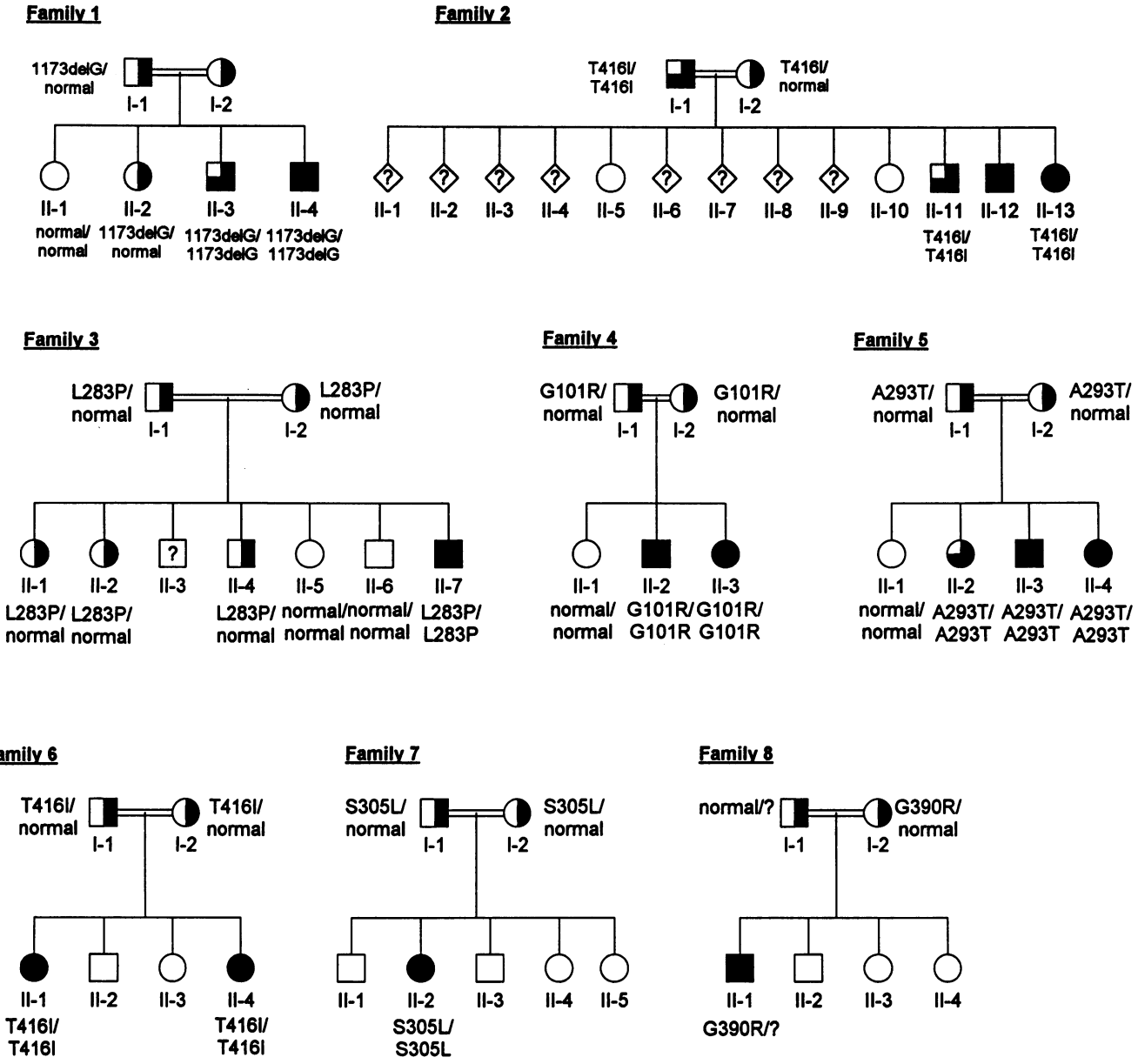

Figure 1 Family pedigrees and genotypes. Asymptomatic patients are represented by ¾-filled symbols.

primers in Greenberg et al. (1995). The entire coding region was covered by these overlapping fragments. For each of the amplification reactions, a total of 35 cycles of amplification were performed, each consists of 1-min denaturation step at 94°C, 1.5-min period at 52°C for annealing, and 2-min primer extension at 70°C. The final extension step lasted 10 min. The two strands of each fragment were sequenced using the dideoxy chain-termination reaction (Sanger et al. 1977) with Sequenase version 2.0 (United States Biochemical, Amersham), and ^{35}S α-dATP. Sequencing reactions were electrophoresed through a 6% denaturating polyacrylamide gel, dried under vacuum, and exposed to X-ray film for 24–36 h.

Sequencing of the entire coding region was performed using the cDNA of individual II-3 of family 1, II-13 of family 2, II-7 of family 3, II-3 of family 4, II-3 of family 5, II-1 of family 6, II-2 of family 7, and II-1 of family 8. In the first-degree relatives, only the relevant cDNA fragments were sequenced. Each base substitution, which was identified at the cDNA of a patient, was also examined at the genomic DNA of the patient and at the cDNA of the parents.

Results

Seven mutations were identified in 15 of the 16 GA-I alleles: a Thr-to-Ile substitution at codon 416 (ACA→

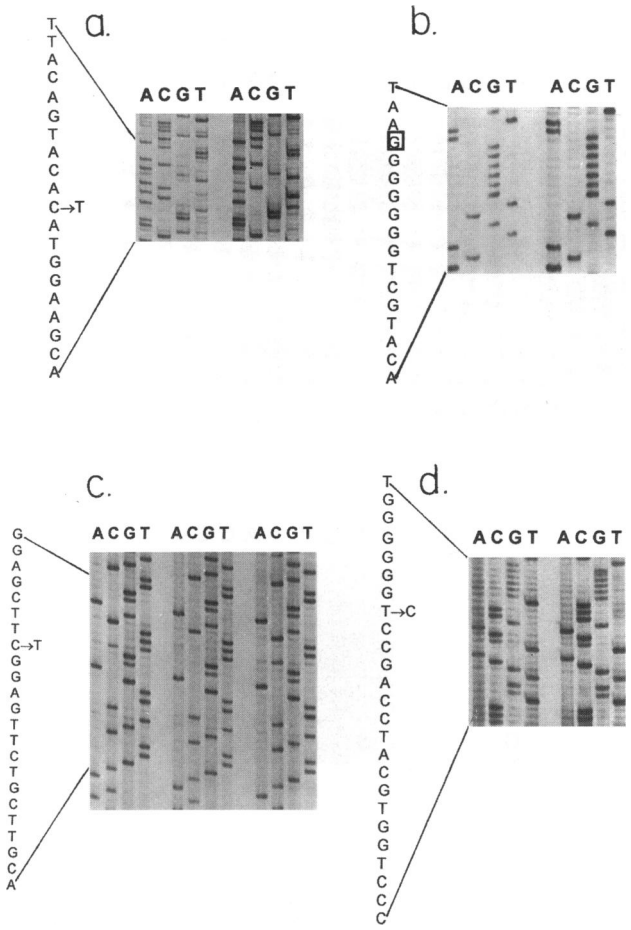

Figure 2 *a,* Sequence analysis of a cDNA fragment of exon 10/exon 11 junction. A sample from the normal control is shown in the left-hand lanes, and a sample from patient II-13 of family 2 who is homozygous for the T416I mutation is shown in the right-hand lanes. The C→T mutation at position 1247 is shown. *b,* Part of exon 10 cDNA. The normal control is shown in the right-hand lanes, and patient II-3 of family 1 who is homozygous for the 1173delG mutation is shown in the left-hand lanes. The deleted nucleotide is inside the box. *c,* Part of exon 8 cDNA. the normal control is shown in the right-hand lanes, patient II-2 of family 7, who is homozygous for the S305L mutation is shown in the middle lanes, and the heterozygous father of family 7 is shown in the right-hand lanes. The C→T mutation at position 914 is shown. *d,* cDNA fragment of exon 7/exon 8 junction. The normal control is shown in the left-hand lanes, and patient II-7 of family 3, who is homozygous for the L283P mutation, is shown in the right-hand lanes. The T→C mutation at position 848 is shown.

ATA), designated "T416I" (four alleles); a Gly-to-Arg substitution at codon 390 (GGG→CGG), designated "G390R" (one allele); a Ser-to-Leu at codon 305 (TCG→TTG) designated "S305L"; an Ala-to-Thr substitution at codon 293 (GCC→ACC), designated "A293T"; a Leu-to-Pro substitution at codon 283 (CTG→CCG), designated "L283P"; a Gly-to-Arg at codon 101 (GGG→AGG), designated "G101R"; and 1-bp deletion, in the cluster of the 7Gs at position 1167-1173, designated "1173delG" (figs. 2 and 3). The latter five mutations were identified in two alleles each. The genotype of the patients and their relatives are presented in table 2 and figure 1.

Mutation G390R was found in the cDNA of patient II-1 of family 8, who seemed to be homozygous for this mutation as well as for the polymorphic substitution at codon 391 (GGG→GGC) designated "G391G." In genomic DNA, this patient and his mother were heterozygous for both changes, indicating that the two base substitutions were carried by the same maternal allele, that the mutated paternal allele is not expressed, and that the investigated cDNA of the patient was derived only from the maternal allele. The G391G polymorphism was also identified in one of the Arab Moslem control samples.

Discussion

Six point mutations and one microdeletion were identified in eight families, six of Arab origin and two of Jewish origin. Seven of the eight families were homozygous for their mutations, as expected by the consanguinity in all families. In each family, only one mutation was identified in the entire coding region, suggesting that these mutations are disease causing; furthermore, the genotype of available first-degree relatives in all families was consistent with their residual enzymatic activity. Patient II-1 of family 8 was a compound heterozygous for the G390R mutation and for another, unidentified, mutation. The allele which carried the unidentified mutation could not be reverse transcribed, indicating that the mutation may interfere with mRNA production or stability.

GCD is one of seven homologous flavoproteins belonging to the acyl-CoA dehydrogenase family. The structure of one of these enzymes, porcine MCAD, was solved by crystallography (Kim and Wu 1988; Kim et al. 1993). In view of marked sequence similarity between human GCD and porcine MCAD, Goodman et al. (1995) tentatively assigned residue E414 of the GCD precursor as the base catalyst that abstracts the α-proton from glutaryl-CoA. Thus, the 1173delG mutation identified in family 1, which changes the correct reading frame starting from residue 392, is expected to manifest by lack of catalytic activity.

Residue T416, which is adjacent to E414, is mutated in family 2 and 6. These two amino acids are part of the YEGT sequence, which is conserved in most FAD-dependent acyl-CoA dehydrogenases. Residue T378 of the mature MCAD is the analogue of T416 of the GCD precursor. In MCAD crystal structure without the substrate (3MDD; Protein DataBank, Brookhaven National Laboratory, NY), residue T378 forms a hydrogen bond with the flavin molecule so that the distance between the oxygen atom of the R-chain of the threonine and the

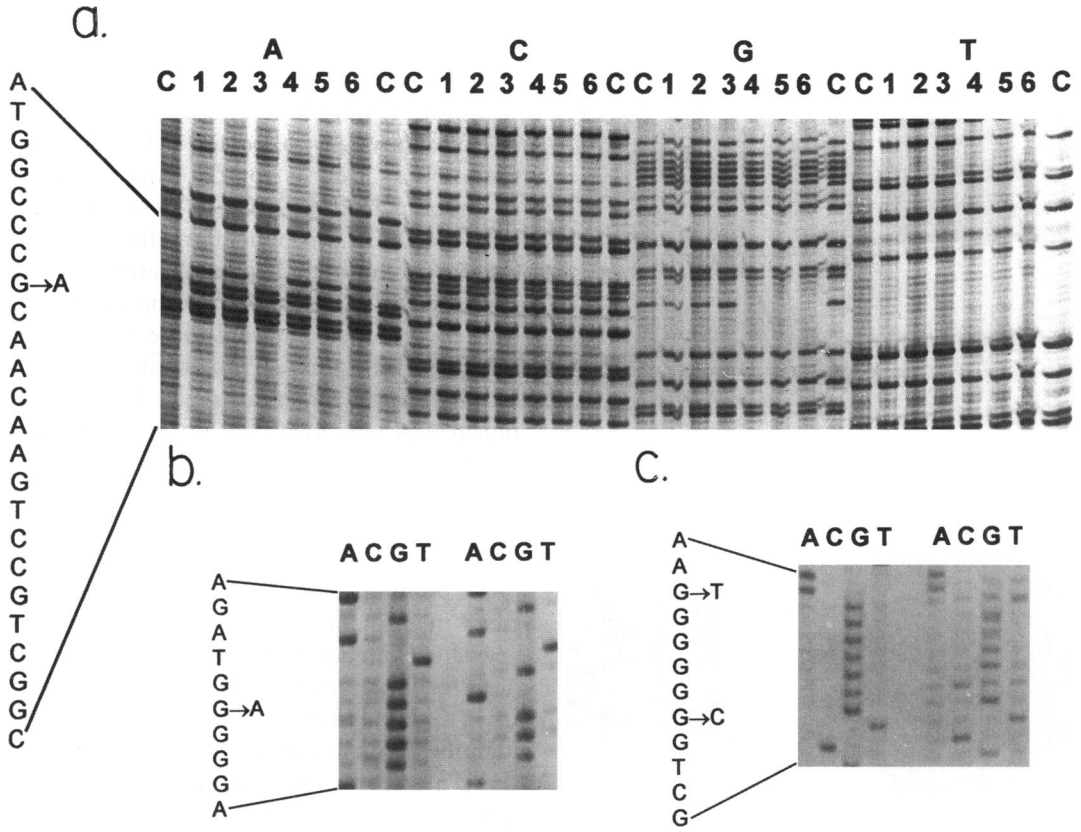

Figure 3 *a*, Part of exon 8 cDNA of family 5. Lane C, control. Lanes 1 and 2, mother and father, who are heterozygous for the A293T mutation. Lane 3, individual II-1, who is healthy homozygote. Lane 4, individual II-2, who is asymptomatic but homozygous for the A293T mutation. Lanes 5 and 6, individuals II-3 and II-4, who are severely affected and homozygous for the A293T mutation. The G→A mutation at position 877 is shown. *b*, Part of exon 4 cDNA. The normal control is shown in the left-hand lanes, and patient II-3 of family 4, who is homozygous for the G101R mutation, is shown in the right-hand lanes. The G→A mutation at position 301 is shown. *c*, Part of exon 10, which was amplified from genomic DNA. The normal control is shown in the left-hand lanes, and patient II-1 of family 8, who is heterozygous for the G390R mutation and the G391G polymorphism, is shown in the right-hand lanes. The G→C mutation at position 1168 and the G→T substitution at position 1173 are shown.

hydrogen atom of the 4′-hydroxyl group of the ribose, is 2.49 Å. FAD is essential not only for the catalytic activity of MCAD but also for the stability of the homotetramer even before the binding of the substrate. In the absence of FAD, the release of the MCAD molecule from its complex with hsp60, and its proper folding into homotetramer within the mitochondria, is impaired (Saijo and Tanaka 1995). Thus, mutation T416I could have serious consequences on the stability of the GCD homotetramer because the amino acid isoleucine is unable to form hydrogen bonds.

Residues 240–280 of the MCAD molecule form one of the α-helices (the helix G), which participates in the anchoring of the CoA moiety of the substrate (Kim et al. 1993). On the basis of a considerable similarity at the flanking regions, it is proposed that helix G of the MCAD molecule corresponds to residues 279–319 of the GCD precursor. Thus, the pathogenic mechanism of the mutations at residues L283, A293, and S305 of the GCD protein in three of our patients may involve interference with substrate binding. Alternatively, the mutations may impair the protein function by altering its folding. In all three mutations, the substituting residue differs markedly from the original one. Leucine, which

Table 2

The New Mutations in the Glutaryl-CoA Dehydrogenase Gene

Position	Mutation	Type	Family No.
1173delG	Frameshift	Deletion	1
1247C→T	T416I	Missense	2, 6
848T→C	L283P	Missense	3
301G→A	G101R	Missense	4
877G→A	A293T	Missense	5
914C→T	S305L	Missense	7
1168G→C	G390R	Missense	8

Table 3

Sequence Similarity around the G390R Mutation

Sequence Name	Sequence
Glutaryl-CoA dehydrogenase	389-L G GNG
Isovaleryl-CoA dehydrogenase	379-F G GNG
Very-long-chain acyl-CoA dehydrogenase	433-M G GMG
Long-chain acyl-CoA dehydrogenase	387-H G GWG
Medium-chain acyl-CoA dehydrogenase (mature)	351-L G GNG
Short-chain acyl-CoA dehydrogenase	366-L G GMG

can form a stable hydrogen bond, is replaced at position 283 by proline, whose cyclic side chain gives rise to steric hindrance and prevents it from forming a regular α-helix and stable hydrogen bonds with the neighboring amino acids; the small, nonpolar, hydrophobic alanine at position 293 is substituted by the larger, polar threonine; and the hydrophilic serine at position 305 is substituted by nonpolar leucine. Similarly, mutation G101R is associated with a drastic substitution; the short, uncharged glycine, with its unique ability to assume many backbone dihedral angles, is replaced by arginine, with its bulky, positively charged R-group. It should be mentioned that the change of polarity caused by the S305L mutation is probably not related to its pathogenic mechanism; alanine, another hydrophobic residue, occurs naturally at position 305 in murine GCD (Koeller et al. 1995). Thus, the pathogenicity of the S305L mutation is possibly derived from the longer side chain of leucine compared to those of serine and alanine.

Residue G390, which is mutated in the patient of family 8, is part of a highly conserved sequence (table 3). Residue G353 of the mature MCAD, corresponding to G391 in the GCD precursor, forms a hydrogen bond with one of the oxygen atoms of the pyrophosphate of the FAD (Kim et al. 1993). The introduction of arginine at position 390 possibly interferes with the binding capacity of residue G391. It is of note that, in the mouse GCD sequence, position 390 is also occupied by glycine and not by arginine (D. Koeller and S. I. Goodman, unpublished data).

The pathogenesis of GA-I has been attributed to depletion of GABA in the basal ganglia as a result of inhibition of its synthetic enzyme, glutamic acid decarboxylase, by excess of glutaric acid (Stokke et al. 1976). Enhanced glutamatergic neurotransmission by interference with glutamate reuptake or by activation of glutamatergic binding sites by high levels of glutaric acid has also been proposed (Goodman et al. 1985). We have previously suggested that the clinical variability in GA-I, observed in family 1 and 2, was the result of an unrelated, genetically polymorphic, protective mechanisms such as decreased vulnerability of glutamic acid decarboxylase or of the glutamatergic networks in the basal ganglia to glutaric acid inhibition (Amir et al. 1989). The results of the present study do not support a molecular basis, such as an alternative mRNA-splicing mechanism, for the clinical variability. First, in families 1, 2, and 5 all the amplified cDNA fragments were of normal size. Second, alternative splicing confined to the brain tissue of the asymptomatic individuals of family 1 and 2 is unlikely, because the skipping of exon 10 or 11, respectively, would interfere with the conserved YEGT sequence.

In five of the families included in this study, the diagnosis of GA-I in the index case was followed by identification of another affected sibling, who was either asymptomatic, suffered from a milder form of the disease, or was misdiagnosed for a nonmetabolic condition. Despite this high rate of underdiagnosis, GA-I is still a rare disease; to the best of our knowledge, the eight families included in this study were the only diagnosed GA-I families in Israel and the former West Bank, ~6 million inhabitants, between the years 1987 and 1994. Similar figures were obtained in Sweden and Norway; over a period of 15 years, 10 GA-I families were identified in a population of 10 million people (Kyllerman et al. 1994).

The Arab population in the Middle East is living mostly within small towns and villages and marry within the kindred; each village may thus be considered a small isolate. There are two possible explanations for the finding of GA-I patients in six Moslem families that originated from different villages. One possibility is that the disease is caused by one mutation that occurred a long time ago and is now frequent in the Moslem population in the region. The second possibility is that multiple novel mutations occurred recently and, because of marriage customs and high consanguinity rate, the mutations are now seen in homozygosity in the affected patients. If GA-I was caused by one common mutation in this population, a very high incidence of the disease would be expected. Since this is not the case, the second alternative appears more attractive and is supported by the finding of a novel, homozygous mutation, in five of the six Moslem families. Similar observations were made for other disorders such as metachromatic leukodystrophy and Hurler disease (Bach et al. 1993; Heinisch et al. 1995). The phenomenon of multiple different mutations in a single gene within a small geographic area seems to be common (Zlotogora et al. 1996).

Acknowledgments

This work was supported in part by the Israeli Ministry of Health (grant 3272-95), the Joint Research Fund of the Hebrew University and Shaare-Zedek Medical Center, and the

Mirsky Foundation. We are grateful to Prof. J. Zlotogora for the critical reading of the manuscript. The expert technical assistance of Edna Chen is acknowledged.

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