Characterization of novel GCDH pathogenic variants causing glutaric aciduria type 1 in the southeast of Mexico

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Abstract

Biallelic mutations of the GCDH gene result in Glutaric Aciduria type 1 (GA1; OMIM #231670), an uncommon autosomal recessive inborn error caused by the deficiency of glutaryl-CoA dehydrogenase (CCDH), a mitochondrial matrix protein involved in the degradation of L-lysine, L-hydroxylysine, and L-tryptophan. The enzymatic deficiency leads to the accumulation of neurotoxins causing macrocephaly at birth, hypotonia and dystonia due to bilateral striatal injury, that evolves with aging, if untreated, to fixed dystonia and akinetic-rigid parkinsonism. In this article, we describe the results of molecular studies of 5 unrelated patients with GA1 in Southern Mexico. Mutational analysis identified 2 novel likely pathogenic GCDH variants (p.Leu130Pro and p.Gly391Val), 1 pathogenic variant that is predicted to cause a premature stop codon (p.Leu370*), and 2 previously reported pathogenic variants (p.Arg294Trp and p.Arg294Gln). The recurrence of the p.Leu130Pro variant (60% of mutant alleles) suggested a possible founder mutation effect. Our results expand the mutational spectrum in GA1 patients and support the importance of early diagnosis through newborn screening that promotes early nutritional treatment and prevents metabolic crisis.

Take home message: Glutaric Aciduria type 1 has a wide mutational spectrum; the p.Leu130Pro variant may be a founder mutation in Southeast Mexico.

1. Introduction

Glutaric aciduria type 1 (GA1; OMIM #231670) is a rare autosomal recessive neurometabolic disorder caused by inactivating biallelic mutations in the glutaryl CoA dehydrogenase gene (GCDH), located at chromosome 19p13.2 [1]. GCDH is a flavin adenine dinucleotide-dependent mitochondrial matrix protein that decarboxylates glutaryl-CoA to crotonyl-CoA and carbon dioxide [2]. GCDH deficiency results in abnormal metabolism of L-lysine (Lys), L-hydroxylysine, and L-tryptophan (Trp), leading to an accumulation of metabolites as glutaric acid (GA), 3-hydroxyglutaric acid (3-OH-GA), gluconic acid, and glutaryl carnitine (C5DC), which induce neuronal death through excitotoxicity, as well as mitochondrial and neurotransmission abnormalities [3]. Other identified mechanisms contributing to GA1 pathogenesis include impairment of brain energy metabolism [4,5], disturbance of neurotransmission [6–8], dysregulation of cerebral blood flow [9], and endothelial cell dysfunction [10].

Clinical manifestations of GA1 patients can vary considerably. Most of them have macrocephaly at birth or rapid increase in head circumference postnatally [11]. Acute encephalopathic deterioration, which are usually originated by febrile disease, vaccinations, or surgical procedures, take place more commonly between 6 and 36 months of age, although it may occur as late as 6 years of age [12,13]. Subsequently, a complex dystonic movement disorder develops accompanied by feeding and speech problems, failure to thrive, recurrent aspiration, immobilization, severe motor deficit, and early death occur [14]. Metabolic treatment, consisting of restricted lysine and tryptophan diet, carnitine supplementation, and intensified emergency treatment during catabolism, is an effective therapy that has shown to improve neurologic outcome in those patients with an early diagnosis [24].

GA1 has a worldwide incidence of 1:110,000 [16]. Since the description of two patients in 1975 [1,15], about of 500 subjects with GA1 have been described worldwide and approximately 200 pathogenic variants in GCDH gene has been reported [17]. Five distinct populations (Amish Community in Lancaster County, PA, USA; the Oji-Cree First Nation in Manitoba Ontario, Canada; the Irish Travellers in the Republic of Ireland; the Lumbee Indian Tribe in North Carolina, USA, and the Xhosa of the South African black population) have been identified...
as having a high GA1 carrier frequency and incidence, up to 1:10 and up to 1:250, respectively [18–22].

In GA1, metabolites as CSDC accumulated in fluids and tissues and can be identified by chromatography/mass spectrometry, which in conjunction with GCDH genetic analysis allows for an early diagnosis for the prevention and reduction of neurological disabilities [23]. Thus, GA1 is considered a treatable condition and guideline-according treatment can reduce considerably the frequency of acute encephalopathic crises and mortality in early diagnosed patients [15]. In Mexico there is no data about the incidence or the genetic basis of GA1, in either symptomatically or pre-symptomatically diagnosed patients.

The aim of this article is to describe the genetic profile of a group of patients diagnosed with GA1 through newborn screening (NBS) in the southeast Mexico. In Mexico, newborn screening is available only through some regional NBS programs and/or the private sector. The patients included in this study belong to the Maya ethnic group, from the Yucatan peninsula in the southeast of Mexico. Five different pathogenic variants, 3 of them novel, were identified, expanding the mutational spectrum of GCDH gene. In addition, a founder effect for the p.Leu130Pro in the Mayan population is proposed.

2. Material and methods

The study was approved by the local Institutional Review Board and investigations were conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all participants. Five unrelated patients with GA1 were studied. All of them were children, originated from the southeast region of Mexico, and were diagnosed through expanded NBS program. The ages and clinical data showed correspond to the last follow-up. NBS was performed by the analysis of dry blood spot with tandem mass spectrometry, with a glutarylcarnitine (C5DC) cut-off of 0.9 μmol/L. Confirmatory test was achieved by qualitative urinary organic acid analysis by GC/MS (Agilent technologies 5977 / Agilent technologies 7820A) on urine samples, searching for an increased excretion of 3-hydroxyglutaric and glutaric acid. Enzyme testing was not performed in our cohort of patients as, to the best of our knowledge, this test is not available in Mexico.

For GCDH genetic analysis, genomic DNA was isolated from peripheral blood leukocytes (patient # 1) or dry blood spot (patients # 2–5) following standard procedures. Polymerase chain reactions were designed to amplify all coding exons and the flanking intronic regions of the GCDH gene. Oligonucleotide sequences and PCR conditions are available on request. Amplification products were analyzed on 1.5% agarose gels and purified. Later, DNA fragments were sequenced by the chain termination method using the Big Dye Terminator version 3.1 Cycle Sequencing Kit. Sequences were compared manually against Ensembl Transcript ID ENS00000222214.10. To establish pathogenicity or likely pathogenicity of variants, the American College of Medical Genetics (ACMG) and Genomics and the Association for Molecular Pathology criteria were considered [24]. After diagnosis, treatment was initiated in all patients, based on restricted lysine and tryptophan diet, oral carnitine (100 mg/kg/day), arginine, and riboflavin supplementation. All parents were instructed with the emergency treatment regime in case of decompensation based on a high calorie and high biological value protein free diet.

The patients were followed up regularly by an experienced multidisciplinary group, including a pediatrician, clinical geneticist and pediatric nutritionist, recording somatometric and biochemical measurements, following the most recent recommendations for GA1 [15].

3. Results

Table 1 shows the main features of the patients.

**Patient # 1**: A one year-7 months old girl, product of a healthy, non-consanguineous couple, was referred for molecular genetic
diagnosis after being identified with elevated CSDC blood levels (5.24 μmol/L; cut-off: 0.9 μmol/L) through expanded newborn screening in a private laboratory and elevated glutaric acid and 3-OH-glutaric acid in urine organic acids. Parents originated from Yucatan State in southeast Mexico. No familial history for genetic or metabolic diseases, congenital malformations, nor intellectual disability was recorded. She was born after an uneventful pregnancy of 40 weeks, macrocephaly documented at birth. At the time, she is under nutritional support based on restricted lysine and tryptophan diet; dose: one third of whole protein requirement is low Lys and Trp formula (Glutarex) and two thirds of intact protein; l-carnitine (100 to 200 mg/Kg/day); riboflavin (100 mg/day) and arginine containing amino acid supplement [15]. This patient has good compliance with treatment and follow-up. Neurologically, the patient has sporadic tremor and normal development. The patient has been under emergency treatment in two occasions because of acute fever and head trauma. No encephalopathy has been documented. Sanger sequencing of the entire GCDH coding region identified compound heterozygosity for two transitions (c.880C > T, and c.881G > A) in exon 9 (Fig. 1A). These variants have already been previously reported [25,26] and predicts the exchange of the same highly conserved amino acid residue (p.Arg294Trp and p.Arg294Gln). Maternal DNA analysis demonstrated heterozygosity for p.Arg294Gln (Fig. 1B) while paternal DNA was not available.

**Patient # 2.** This 3-year-old girl was product of a healthy, non-consanguineous couple. She was referred for molecular genetic analysis after being identified with elevated CSDC blood levels (1.82 μmol/L; cut-off: 0.9 μmol/L) through the expanded newborn screening state program and elevated glutaric acid and 3-OH-glutaric acid in urine organic acids. Parents originated from Yucatan State in southeast Mexico. No familial history for genetic or metabolic diseases, congenital malformations, nor intellectual disability was recorded. She was born after an uneventful pregnancy of 40 weeks with macrocephaly being documented at birth. Currently, she is under nutritional treatment, based on restricted lysine and tryptophan diet; dose: one third of whole protein requirement is low Lys and Trp formula (Glutarex) and two thirds of intact protein; l-carnitine (100 to 200 mg/Kg/day); riboflavin (100 mg/day) and arginine containing amino acid supplement [15]. This patient has good compliance with treatment and follow-up. Neurologically, the patient had delayed development that corrected with physical therapy. She has been under emergency treatment in one occasion because of acute gastroenteritis. No encephalopathy has been documented. Sanger sequencing of the GCDH gene demonstrated a homozygous c.389T > C transition (Fig. 1C), a variant predicting a p.Leu130Pro missense substitution. This gene variant is considered as likely pathogenic by ACMG due to 3 moderate (PM2, PM3, and PM6) and 3 support criteria (PP2, PP3, and PP4). Parental DNA samples were not available.

**Patient # 3.** This 2-year-aged girl is product of a healthy, non-consanguineous couple and was referred for molecular genetic testing after being identified with elevated CSDC blood levels (4.84 μmol/L; cut-off: 0.9 μmol/L) through the expanded newborn screening state program as well as elevated glutaric acid and 3-OH-glutaric acid in urine organic acids. Parents originated from Yucatan State in southeast Mexico. No familial history for genetic or metabolic diseases, congenital malformations, nor intellectual disability was recorded. She was born after an uneventful pregnancy of 37 weeks. Macrocephaly was identified at birth. Currently, she is under nutritional treatment, that started at diagnosis and is based on restricted lysine and tryptophan diet (Glutarex); dose: one third of whole protein requirement is low Lys and Trp formula and two thirds of intact protein; l-carnitine (100 to 200 mg/Kg/day); riboflavin (100 mg/day) and arginine containing amino acid supplement [15]. This patient had delayed development that corrected with physical therapy. She has been under emergency treatment in two occasions because of fever and respiratory infections. No encephalopathy has been documented. Sanger sequencing of the entire GCDH coding region demonstrated compound heterozygosity for the novel c.389C > T and c.1108delC 1-bp deletion, at exon 11 (Fig. 1E–F). These gene variants have not been previously reported and they are not listed in public databases. The c.1108delC 1-bp deletion is predicted to cause the immediate introduction of a premature stop codon (p.Leu370*).

**Patient # 4.** This girl presented at an age of 2 years. She was product of a healthy, non-consanguineous couple and was referred for genetic testing after being identified with elevated CSDC blood levels (3.24 μmol/L; cut-off: 0.9 μmol/L) through expanded newborn screening state program and elevated glutaric acid and 3-OH-glutaric acid in urine organic acids. Parents originated from Yucatan State in southeast Mexico. No familial history for genetic or metabolic diseases, congenital malformations, nor intellectual disability was recorded. She was born after an uneventful pregnancy of 38 weeks with macrocephaly being observed at birth. Treatment under nutritional support started at diagnosis and is based on restricted lysine ant tryptophan diet (Glutarex); dose: one third of whole protein requirement is low Lys and Trp formula and two thirds of intact protein; l-carnitine (100 to 200 mg/Kg/day); riboflavin (100 mg/day) and arginine containing amino acid supplement [15], but with poor compliance due to socio-demographic factors. Neurologically, the patient had delayed development and myoclonic jerks treated with physical therapy. She has been under emergency treatment in two occasions because of acute gastroenteritis and febrile crisis, with an episode of encephalopathy. At 1 year of age, she developed a metabolic crisis with subsequent neurologic damage. Sanger sequencing of the GCDH gene demonstrated homozygosity for a c.389T > C transition, a variant predicting a p.Leu130Pro missense change at the protein level (Fig. 1G). This variant was aforementioned.

**Patient # 5.** This girl presented at the age of 4 years. She was product of a healthy, non-consanguineous couple and was referred for molecular genetic diagnosis after being identified with elevated CSDC blood levels (4.84 μmol/L; cut-off: 0.9 μmol/L) through the expanded newborn screening state program and elevated glutaric acid and 3-OH-glutaric acid in urine organic acids. Parents originated from Yucatan State in southeast Mexico. No familial history for genetic or metabolic diseases, congenital malformations, nor intellectual disability was recorded. She was born after an uneventful pregnancy of 37 weeks. Macrocephaly was identified at birth. Currently, she is under nutritional treatment, that started at diagnosis and is based on restricted lysine ant tryptophan diet (Glutarex); dose: one third of whole protein requirement is low Lys and Trp formula and two thirds of intact protein; l-carnitine (100 to 200 mg/Kg/day); riboflavin (100 mg/day) and arginine containing amino acid supplement [15]. This patient has good compliance with treatment and follow-up. Neurologically, the patient had delayed development that corrected with physical therapy. She has been under emergency treatment in two occasions because of fever and respiratory infections. No encephalopathy has been documented. Sanger sequencing of the entire GCDH coding region identified compound heterozygosity for the novel c.389C > T and c.1172G > T variants, at exons 6 and 11, respectively (Fig. 1H–I). The c.1172G > T transversion predicts the novel p.Gly391Val missense mutation which is classified as likely pathogenic by ACMG due to 2 moderate (PM2, and PM6) and 2 support criteria (PP2, and PP3).

4. Discussion

GAI is a rare inherited metabolic disorder, arising from biallelic mutations in GCDH, with an estimated overall incidence of 1 in 106,900 [16]. We observe a high incidence of GAI: 5 cases in 10 years , with a birth rate of 34,981 newborns per year, yielding a prevalence of 1:36,442. Most patients are from rural areas, supposedly endogamic [27], that could explain the high prevalence of a specific pathogenic variant, and the possible founder effect. There is no mutational hotspot in GCDH and almost every pathogenic variant is family or population specific. In our study, we found 5 different pathogenic variants (Table 2), 3 of them non-previously reported, being c.389T > C,
Fig. 1. Genetic analysis in GA1 patients.
A: Partial DNA sequencing of GCDH exon 9 showing compound heterozygosity in the patient #1 (c.880C > T and c.881G > A).
B: Heterozygous state in the carrier mother of patient #1 (c.881G > A).
C: Partial DNA sequencing of GCDH exon 6 showing the homozygous c.389T > C mutation in the patient #2.
D: Control exon 6 DNA sequence.
E, F: Partial DNA sequencing of GCDH demonstrating a compound heterozygous state in patient #3: Heterozygous c.389T > C, at exon 6 (E) and heterozygous c.1108delC in exon 11 (F).
G: Partial DNA sequencing of GCDH exon 6 showing the homozygous c.389T > C mutation in the patient #4.
H, I: Partial DNA sequencing of the GCDH showing a compound heterozygous state in patient #5: Heterozygous c.389T > C, at exon 6 (H) and heterozygous c.1172G > T, at exon 11 (I) are shown.
p.Leu130Pro the most frequent variant as it was observed in 6 out of 10 analyzed alleles. The variants p.Arg294Tnp and p.Arg294Gln were previously reported in patients with confirmed GA1 [25,26].

All analyzed patients are alive and under treatment following the proposed recommendations for GA1 management [15] and show a proper response for nutritional treatment. Patient # 3, carrying the p.Leu130Pro variant, is also under medical and clinical follow-up. This patient had an affected brother exhibiting a similar phenotype. Although the establishment of a genotype-phenotype correlation in GA1 has been attempted, the severity of the clinical phenotype seems to be closely linked to the development of encephalopathic crises [28] and to the quality of guideline-adherent treatment [15] rather than to residual enzyme activity or genotype.

To our knowledge this is the first study of GA1 genotypification in Mexico and Latin America and our results provide preliminary evidence of the genetic and clinical features of GA1 in this population. Since it is well known that early treatment enhance neurological prognosis in GA1 [11,12,13], those are also presumed to be due to endogamy [17]. However, in the ancient Mayan population, that is well known, the early treatment of patients with GA1 type 1 is crucial for opportunistic detection of affected individuals. It is possible that the most frequent allele, the p.Leu130Pro, originated from a founder effect in the ancient Mayan population, that is well known. Initial treatment of the clinical phenotype seems to be closely linked to the development of encephalopathic crises [28] and to the quality of guideline-adherent treatment [15].

In conclusion, this work expands the molecular spectrum of glutaric aciduria by describing novel GCDH pathogenic variants.

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