# L-2-Hydroxyglutaric aciduria in two Palestinian siblings with a novel mutation in the *L2HGDH* gene

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## Abstract

*L-2-Hydroxyglutaric aciduria is a rare autosomal recessive neurometabolic disorder caused by deficiency of L-2-hydroxyglutarate dehydrogenase. This enzyme catalyses the conversion of L-2-hydroxyglutarate to alpha-ketoglutarate and its deficiency causes accumulation of L-2-hydroxyglutarate which is s toxic to the brain leading to the leukoencephalopathy.*

*The researchers reported a novel mutation in the L2HGDH gene in two siblings with L-2-Hydroxyglutaric aciduria and described clinical phenotype. The symptoms were presented with developmental delay, cerebellar ataxia, tremor and speech regression. Urine organic acid analysis revealed massive excretion of 2-Hydroxyglutaric acid. Brain magnetic resonance imaging showed the characteristic leukodystrophy involving the subcortical cerebral white matter and dentate nucleus, sparing the basal ganglia. Genetic analysis of L2HGDH gene showed that they were homozygous for the novel mutation chr14:50,750, 731 c.560_561insATTG (p.C187XfsX1) in exon 5 of L2HGDH gene. Both patients showed symptomatic response to riboflavin and levocarnitine therapy.*

*Conclusion: This report further expanded the genetic spectrum of L-2-HGA and suggested a successful response to treatment with levocarnitine and riboflavin*

***Keywords: L-2-Hydroxyglutaric aciduria, L2HGDH gene, white matter, leukoencephalopathy, ataxia***

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## Introduction

L-2-Hydroxyglutaric aciduria (L-2-HGA) is a rare autosomal recessive neurometabolic disorder affecting mainly the central nervous system. It is caused by mutations in *L2HGDH* gene that encodes L-2-hydroxyglutarate dehydrogenase (Augoustides-savvopoulou et al, 2007; Kranendijk et al, 2012). This enzyme catalyses the conversion of L-2-hydroxyglutarate to alpha-ketoglutarate (Van Schaftingen et al, 2009). L-2-hydroxyglutarate is toxic to the brain, causing a leukoencephalopathy and increases the susceptibility to develop tumors (Van Schaftingen et al, 2009; Steenweg et al, 2009).

Typically, the disease follows a progressive neurodegenerative course. Common features include cerebellar ataxia, mental retardation and learning difficulties (Sass et al, 2008). Other frequently reported manifestations include macrocephaly, seizures, dysarthria and pyramidal and extrapyramidal signs (Sass et al, 2008; Larnaout et al, 2008).

The combination of the involvement of the ubcortical cerebral white matter MRI abnormalities that spare the deep white matter (WM) and the corpus callosum, in addition to the involvement of dentate nuclei and atrophy of the cerebellar vermis has been considered pathognomonic for L-2-HGA (Sass et al, 2008; Fourati et al, 2016).

Currently, 86 mutations in *L2HGDH* gene have been described in 164 individuals, The majority of which are missense mutations that alter invariably conserved amino acids (Kranendijk et al, 2012; Sass et al, 2008).

The researchers reported two siblings diagnosed with L-2-HGA and presenting with the classical phenotype and the characteristic leukoencephalopathy that was limited to subcortical white matter and dentate nucleus. They were homozygous for the novel mutation chr14:50,750,731 c.560_561insATTG (p.C187XfsX1*)* in exon 5 of *L2HGDH* gene which is a duplication mutation of ATTG at codon 187 that results in frame shift and premature termination of protein translation at the amino acid residue 187. Family segregation studies of the *L2HGDH* gene and direct sequencing were performed on the DNA extracted from peripheral blood samples (Augoustides-savvopoulou et al, 2007; Sass et al, 2008) showed that both parents and two brothers were heterozygous for the same mutation and two sisters were homozygous for the wild type allele.

The researchers hypothesized a possible correlation between the genotype, phenotype and the extent of leukoencephalopathy in our patients, but the correlation between genotype and phenotype required more future studies.

This study was approved by the ethics committee of Makassed Hospital and written informed consent was obtained from the parents granting permission for genetic analysis, brain MRI imaging and publication.

## Case Presentation

### Case 1

A 15-year-old male who was the third child of four offsprings of healthy consanguineous parents (first cousins). He was born at term after normal pregnancy and labour. Birth weight was 3.5 kg (+1 SD), length and head circumference at birth were not recorded. The patient's clinical course was uneventful apart from speech delay that was noted after the age of two years. After the age of six years, he developed progressive mental deterioration, ataxic gait, truncal ataxia, hand tremor and interrupted slow speech. Several anticonvulsant medications were given, but with no improvement. Brain MRI performed at the age of eight years and 11years revealed leukodystrophic changes which were not further investigated.

He was hospitalized at the center at the age of 15 years for the evaluation of mental retardation, ataxia, tremor and progressive difficulty of speech. His weight was 46 kg (-1.37 SD), height 160 cm (-1.3 SD) and head circumference 54 cm (-0.65 SD). Neurological examination revealed ataxic gait, dysarthria, dysmetria, dysdiadochokinesia, hand tremor, lower limb spasticity and exaggerated deep tendon reflexes in both lower limbs with ankle clonus. Metabolic workup included: Plasma ammonia 30 µmol/L (normal 11-50 µmol/L), lactate 1.7 mmol/L (normal 0.5-2.2 mmol/L), homocysteine 4 µmol/L (normal < 20 µmol/L). Urine organic acid analysis revealed marked excretion of 2-HGA (qualitative). Plasma amino acids including plasma lysine were normal. Other laboratory findings included normal thyroid function tests, plasma uric acid, kidney and liver function tests. Brain MRI images showed high signals involving subcortical white matter and dentate nucleus (Figure 1). Treatment included levocarnitine orally 75 mg/kg/day and riboflavin orally100 mg three times daily.

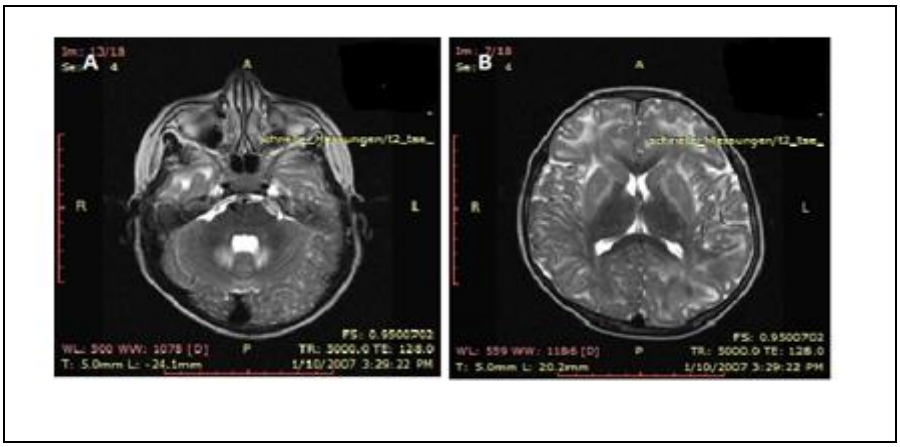


**Figure 1: (A) Axial T2-weighted MR images of the brain of patient 1 showing high signal intensity of the dentate nuclei sparing the brainstem and cerebellar white matter, a finding that is characteristic of L2-OH-glutaric aciduria. (B) Bilateral confluent areas of high signal intensity in the subcortical white matter and subcortical U-fibers sparing the deep white matter of the periventricular region, corpus callosum and internal capsule.**

**Case 2**

An eight-year-old female, the younger sister of patient 1, was also born at term after normal pregnancy and labour. Birth weight was 3.5 kg (+1 SD), length and head circumference were not recorded. The patient's clinical course was characterized by speech delay noted at the age of two years. At the age of three years, she developed truncal ataxia and hand tremor. First hospitalization was at the age of seven years for the evaluation of progressive mental deterioration, hyperactivity and worsening of ataxia and tremor noted after the age of six years.

Her weight was 30 (+1.44 SD), height 130 cm (+ 1.4 SD) and head circumference 52 cm (+0.47 SD). Neurological examination revealed ataxic gait, dysarthria, dysmetria and exaggerated deep tendon reflexes in both lower limbs. Metabolic workup included: Plasma ammonia 25 µmol/L (normal 21-50 µmol/L), lactic acid 2.4 mmol/L (normal 1-2.4 mmol/L). Plasma amino acids including lysine were normal. Urinary organic acid assay also revealed marked excretion of 2-HGA (qualitative). T2-weighted brain MRI also showed high signals involving the subcortical white matter and dentate nucleus (Figure 2). Treatment included Levocarnitine orally 100 mg/kg/day and riboflavin orally 100 mg three times daily.

The family reported improvement in ataxia and speech in both patients after six months of therapy, but the parents denied re-hospitalization for clinical and biochemical evaluation.

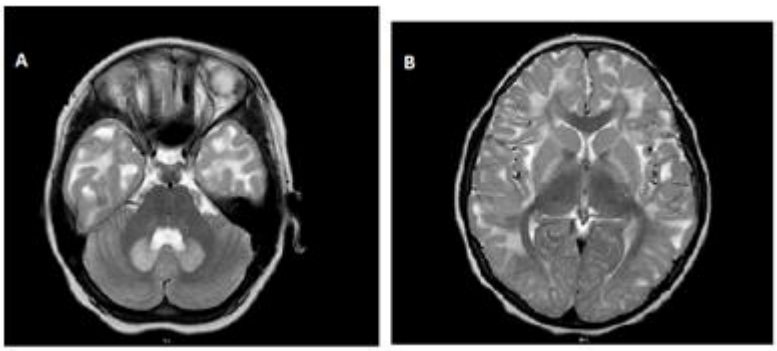


**Figure 2: (A) Axial T2-weighted MR images of the brain of patient 2 showing high signal intensity of the dentate nuclei sparing the brain stem and cerebellar white matter. (B) Bilateral confluent areas of high signal intensity in the subcortical white matter, with the involvement of subcortical U-fibers sparing the deep white matter of the periventricular region, corpus callosum and internal capsule.**

## Methods and Materials of DNA sequencing

**DNA extraction**: Genomic DNA was extracted from whole peripheral venous blood, using a commercially available kit (MasterPure™ Complete DNA & RNA Purification Kit, Cat No.MCD85200).

**Polymerase chain reaction (PCR):** Polymerase chain reaction amplification and DNA sequencing were used to screen exon 5 to detect the presence of the mutation in this family.

A total reaction 50µl for PCR containing; 25µl of ready mix GO Taq green master mix (Promega), 0.1 µg/µl of genomic DNA, 4% primers (F/R) and water. DNA amplification was done by using these primers L2HGDH-5F: gaagaaaagcttggcaaaatc and L2HGDH-5R: caaaacccatggatatggag. The PCR cycling conditions included preheating for 5 minutes at 95°C, followed by 35 cycles of 95°C for 30 seconds annealing at 60°C for 30 seconds 72°C for 30 seconds and a final extension 5 minutes at 72°C.

**DNA Sequencing**: DNA sequencing was performed on an ABI sequencer and the results were compared to the wild type sequence (accession: NG_008092.1) using the Mutation Surveyor software.

The result of the molecular genetic analysis showed that both patients were homozygous for the mutation chr14:50,750,731 c.560_561insATTG (p.C187XfsX1*)* in exon 5 of *L2HGDH* gene. Family segregation studies showed that both parents and two brothers were heterozygous for the same mutation and two sisters were homozygous for the wild allele.

The c.560_561insATTG mutation is in exon 5 of the L2HGDH gene is an insertion and duplication of the ATTG nucleotides at codon 187 that leads to frame shift and premature termination of protein translation (p.C187XfsX1).

The researchers believe that this variant is a pathogenic mutation because it leads to a frameshift with premature termination of protein synthesis after one codon (Figure 3). In addition, the researchers tested the pathogenicity of this variant by Mutation Tasters software and the prediction was that it is a disease causing mutation. Furthermore, the analysis of the Human Genome Database (HGD) indicated that there was a frameshift mutation that was reported in the database at an adjacent amino acid.

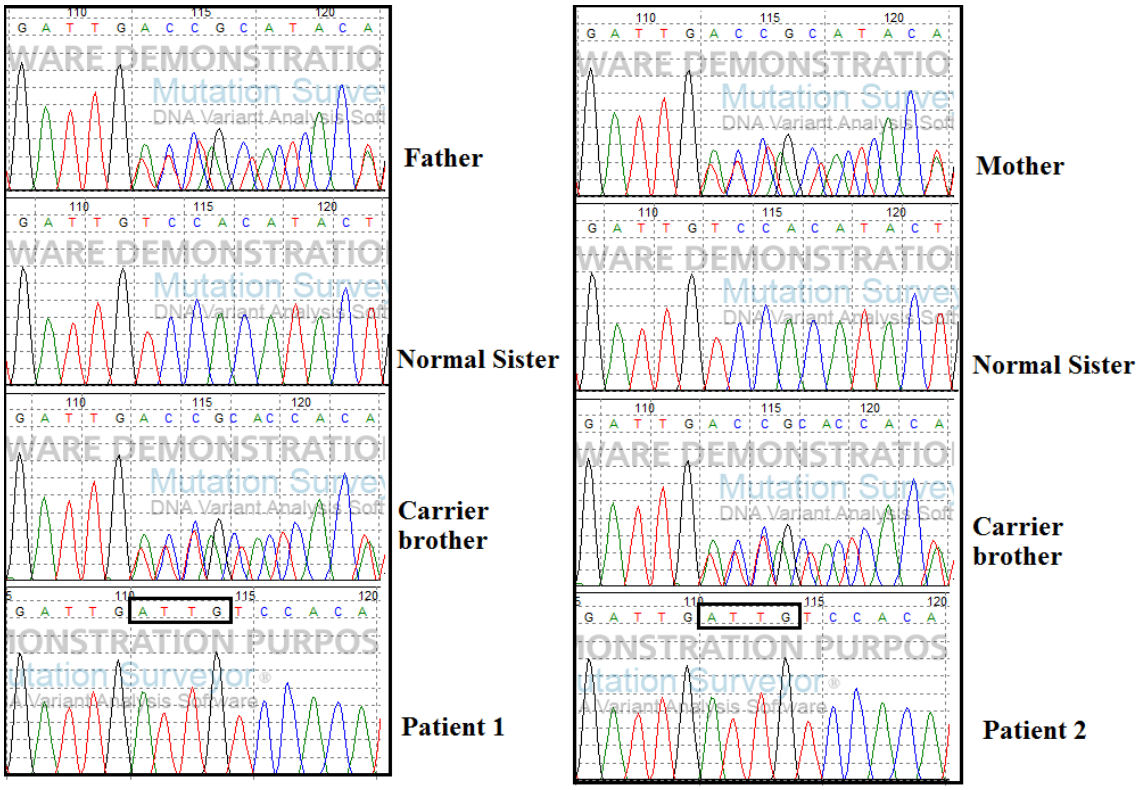


**Figure 3: Mutation analysis: Direct sequencing and segregation study of L2HGDH (exon5) for the family showed that both patients were homozygous for the novel mutation c.560_565insATTG (p.C187XfsX1), the parents and two healthy brothers were heterozygous for the same mutation while the two healthy sisters were homozygous for the wild allele.**

## Discussion

The two siblings in our report were homozygous for the novel mutation chr14:50,750,73 c.560_561insATTG (p.C187XfsX1*)* in exon 5 of *L2HGDH* gene. This genotype correlated with the phenotype, leukodystrophy on brain MRI and the biochemical finding of increased urine level of 2-HGA in both patients. Both patients had identical phenotype consisting of cerebellar ataxia, pyramidal syndrome and mental retardation. They also had white matter MRI abnormalities that were limited to subcortical white matter and the dentate nuclei.

Molecular genetic heterogeneity in the *L2HGDH* gene was reported in a series of 106 patients belonging to 83 families (Steenweg et al, 2010). The study reported 53 mutations, 35 of which were novel, and the majority were missense variants that invariably altered conserved amino acids. The main manifestations were developmental delay, cerebellar ataxia, epilepsy and macrocephaly.

Characteristically, in patients with progression of the disease, WM changes and basal ganglia signal involvement became more diffuse resulting in atrophy of the cerebral WM atrophy (Steenweg et al, 2009; Fourati et al, 2016). A retrospective study of 14 brain MRI studies performed on 10 patients with L-2-HGA showed extensive confluent subcortical signals with symmetrical involvement of dentate nuclei and basal ganglia (Sudhakar et al). In the report, patient 1 did not show spread of WM abnormalities at the age of 16 years and both patients did not show the involvement of basal ganglia. This finding might represent a genotype-phenotype correlation, or represent one end of the spectrum of MRI abnormalities in this disorder. However, because there were so many different genotypes, it was not possible to define a specific phenotype associated with each mutation.

Treatment of a 40-year –old female with L-2-HGA who had limb spasticity, tremor, severe dystonia involving the neck and arms, and mild cognitive delay with levocarnitine and FAD resulted in improvement of dystonia and tremor and normalization of gait and decreased urinary excretion of L-2-Hydoxyglutaric acid after six months of treatment.

The clinical improvement was maintained for more than four years after the start of therapy (Samuraki et al, 2008). Successful therapy was also reported with riboflavin (100 mg daily) in a 16-year-old boy resulting in partial improvement of cognitive and motor function within days of treatment. Decreased excretion of L-2-hydroxyglutaric acid was noted after three months of treatment (Yilmaz et al, 2009). Interruption of riboflavin treatment was followed by the recurrence of the symptoms and increased urinary excretion L-2-hydroxyglutaric acid.

In contrast to these reports, a nine-year-old female with L-2-HGA developed progressive tremor, ataxic gait, dysarthria and mental retardation despite treatment with riboflavin (200 mg/day) (Jequier et al, 2008). She had persistent L-2-hydroxyglutaric acid in body fluids after two months of treatment. Her sister died suddenly at the age of 11 months during intercurrent illness. Both were homozygous for a splice site mutation in *L2HGDH* gene.

The patients showed partial improvement of ataxia and speech after six months of treatment with levocarnitine and riboflavin but unfortunately re-hospitalization for evaluation of neurological status and biochemical investigations was denied.

Although the researchers reported a novel mutation in the *L2HGDH* gene, the small sample size was the main limitation of the study which made genotype-phenotype correlation difficult

## Conclusion

The report further expanded the genetic spectrum of L-2-HGA. It also suggested a successful response to treatment with levocarnitine and riboflavin. Whether the response was sustained and correlated to the genotype and WM abnormalities, it still needs to be further studied in future reports.

## Conflict of interest

The authors declare that there is no conflict of interest.

## Funding

There was no fund for this study.

## Consent for publication

Written informed consent was obtained from the probands' parents for publications of their clinical details.

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# حموضة ل-2-هيدروكسيغلوتاريك أسيد في أخوين فلسطينيين وتسجيل طفرة وراثية فريدة في *L2HGDH* gene

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**ملخص**

*حموضة ل-2-هيدروكسيغلوتاريك أسيد، هي مرض وراثي نادر من أمراض الأيض التي تصيب الدماغ، ناتج عن نقص خميرة ل-2--هيدروكسيغلوتاريت دهيدروجينيز، الذي يحول ل-2-هيدروكسيغلوتاريت إلى ألفا-كيتوغلوتاريت. فتراكم حامض ل-2-هيدروكسيغلوتاريت سام لخلايا الدماغ، ويؤدي إلى ضرر خلايا الدماغ وأنسجته.*

*لقد سجلنا طفرة جينية - لأول مرة - لأخوين، بهذا المرض. ووصفنا الحالة السريرية لهما. وعلامات المرض تمثلت بتأخر النمو، وترنح ناتج عن خلل في المخيخ، ورجفة في اليدين، وتراجع مضطرد في النطق. وفحص الأحماض العضوية في البول، أظهر زيادة في إفراز حامض 2-هيدروكسيغلوتاريت. والرنين المغناطيسي للدماغ أظهر خللاً في المادة البيضاء تحت قشرة الدماغ والنواة المسننة، دون إصابة النواة القاعدية في الدماغ. وتحليل الجين المسبب للمرض أظهر إصابتهما بطفرة، تسجل - للمرة الأولى - في القطعة الخامسة من الجين.*

*والمريضان أظهرا استجابة لعلاج الرايبوفلافين والكارنتين، وتحسن في الأعراض.*

*والنتيجة: هذا البحث يزيد عدد الطفرات الجينية المسجلة لهذا المرض، كما يشير إلى استجابته للعلاج بدواء الرايبوفلافين والكارنتين.*

***الكلمات الدالة: حموضة ل-2-هيدروكسيغلوتاريك، جين L2HGDH ، المادة البيضاء، اعتلال بيضاء الدماغ، اختلاج الحركة.***

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