original article

Clinical, neuroimaging, and genetic features of L-2-hydroxyglutaric aciduria in Arab kindreds

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Ann Saudi Med 2014; 34(2): 107-114

DOI: 10.5144/0256-4947.2014.107

BACKGROUND AND OBJECTIVES: L-2-hydroxyglutaric aciduria is a neurometabolic disorder with autosomal recessive mode of inheritance in which patients exhibit elevated L-2-hydroxyglutaric acid in body fluids, central nervous system manifestations, and increased risk of brain tumor formation. Mutations in L2HGDH gene have been described in L-2-hydroxyglutaric aciduria patients of different ethnicities. The present study was conducted to perform a detailed clinical, imaging and genetic analysis.

DESIGN AND SETTINGS: A cross-sectional clinical genetic study of 16 L-2-hydroxyglutaric aciduria patients from 4 Arab consanguineous families examined at the metabolic clinic of the hospital.

PATIENTS AND METHODS: Genomic DNA was isolated from the blood of 12 patients and 10 unaffected family members, and the L2HGDH gene was sequenced. DNA sequences were compared to the L2HGDH reference sequence from GenBank.

RESULTS: All patients exhibit characteristic clinical, biochemical, and imaging features of L-2-hydroxyglutaric aciduria, and 4 patients exhibited increased incidence of brain tumors. The sequencing of the L2HGDH gene revealed the c.1015delA, c.1319C>A, and c.169G>A mutations in these patients. These mutations encode for the p.Arg339AspfsX351, p.Ser440Tyr, and p.Gly57Arg changes in the L2HGDH protein, respectively. The c.169G>A mutation, which was shown to have a common origin in Italian and Portuguese patients, was also discovered in Arab patients. Finding of the homozygous c.159T SNP associated with the c.169G>A mutation in Arab patients points to an independent origin of this mutation in Arab population.

CONCLUSION: The detailed description of clinical manifestations and L2HGDH mutation in this study is useful for diagnosis of L-2-hydroxyglutaric aciduria in Arab patients. While reoccurrence of an L2HGDH mutation in L-2-hydroxyglutaric aciduria patients of different ethnicity is extremely rare, the c.169G mutation has an independent origin in Arab patients. It is likely that this mutation may also be present in patients of other ethnicities.

L-2-hydroxyglutaric aciduria (OMIM #236792) is a neurometabolic disorder with an autosomal recessive mode of inheritance. Affected individuals have elevated levels of L-2-hydroxyglutaric acid in the urine and other body fluids (including cerebrospinal fluid and plasma).[1] The disease manifests during infancy or early childhood, but sometimes it may present in adulthood with milder phenotypes.[2] Patients exhibit characteristic clinical features, which include psychomotor function regression beginning in infancy, speech delay, severe mental retardation, pyramidal and extrapyramidal signs, demyelination of white matter, cerebral and cerebellar atrophy, seizures, leukoencephalopathy, and other neurologic manifestations.[3] In some patients, this disorder leads to the formation of brain tumors.[4] The brain tumors described in the published studies with L2HGDH are medulloblastoma, primitive neuroectodermal tumors, and fibrillary astrocytoma.[5]

The precise cause of higher incidence of brain tumor in this condition is unknown. This metabolic disorder is caused by mutations in the L-2-hydroxyglutarate dehydrogenase (L2HGDH) gene at chromosome 14q22.1, which encodes for the L-2-hydroxyglutarate dehydrogenase.[6-10] The L2HGDH gene is expressed in multiple tissues with the strongest expression in the brain.[6] The L-2-hydroxyglutarate dehydrogenase catalyzes the oxidation of L-2-hydroxyglutaric acid to 2-ketoglutaric acid. It was suggested that the pathologic phenotypes of patients result from the toxic effects of L-2-hydroxyglutaric acid on central nervous system.[11]

The present study describes the clinical presentation and mutation analysis of the L2HGDH gene of L-2-hydroxyglutaric aciduria patients from four consanguineous Arab families.

PATIENTS AND METHODS

Patients

The four Arab families with 16 L-2-hydroxyglutaric aciduria patients originated from different regions of Saudi Arabia. Their clinical data and family details were gathered during their visits to the Metabolic Clinic. L-2-hydroxyglutaric aciduria was diagnosed on the basis of elevated levels of L-2-HG in urine. The urine specimens of patients from four families with clinical suspicion of metabolic diseases were analyzed for organic acids by gas chromatography-mass spectrometry. The urinary L-2-HG concentration was determined according to Struys et al.[12] The data related to patients V-6 (family 2) and IV-1 (family 4) was not available. Their clinical histories were mentioned in the reports of their family members. Consent was obtained from patients or their legal guardians. The internal review board approved the study.

Mutation analysis

Blood samples from 12 patients and 10 unaffected family members were collected in ethylenediaminetetraacetic acid–containing tubes and DNA was extracted using the automated MagNA pure LC system (Hoffmann-La Roche Ltd). The 10 exons of the L2HGDH gene and their exon/intron boundaries were amplified by polymerase chain reaction using the primer sets described in the supplementary **Table 1**. All amplicons were sequenced on Applied BioSystems Sequencers 3100/3730 (Foster City, CA, USA). The sequences were compared with the reference sequence ENSG00000087299 from the Ensembl genome browser (*http://www.ensembl.org*).

RESULTS

Clinical, biochemical, and imaging features

In this study, 11 males (68%) and 5 females (32%) from 4 consanguineous families were affected with L-2-hydroxyglutaric aciduria. The age range at inclusion in the study was from 8 to 28 years with the mean of 16.2 years. Clinical histories of 13 patients were available for our review (**Figure 1 and Table 1**). For 3 affected subjects whose clinical reports were not available, information was collected from the clinical records of their affected siblings and confirmed by interviews with their unaffected family members. All patients had high concentrations of L-2-hydroxyglutaric acid in the urine, which ranged from 1242 to 5435 mmol/mol creatinine. In 8 unaffected Arab individuals, urine L-2-hydroxyglutaric acid levels ranged from 0.9 to 19.6 mmol/mol creatinine (reference range is 1.3–19 mmol/mol creatinine). The patients exhibited neurological symptoms in the form of speech defects, early developmental delays, affected gait (mostly ataxia), seizures, mental retardation, and various other manifestations (**Table 1**). The most common clinical findings in these patients were affected gait, seizures, speech defects, and developmental delays. The onset of seizures ranged from 4 months to 3 years, with variable frequencies. In addition to above-mentioned neurological manifestations, 4 patients died of brain tumors (family 4: III-3, IV-1, and III-6; family 1: VI-9). The girl VI-9 from family 1, who exhibited developmental delay, speech and gait similar to her brothers, died of brain tumor soon after diagnosis at the age of 8 years. The subject III-3 from family 4 also exhibited developmental delay and died of brain tumor at the age of 18 years, soon after his diagnosis. Metabolic screening and genetic analyses were not done on these 2 individuals. The boy IV-1 from family 4, whose 2 maternal uncles and 2 aunts (1 aunt developed brain tumor family IV, III-6) were affected with L-2-hydroxyglutaric aciduria, also developed brain tumor at the age of 8 years. No clinical information was available for this child.

Occipital frontal circumference (OFC) was available for 14 patients, which included >95th percentile in 4 patients (28.5%), 5th percentile in 1 patient, and normal in the rest of the patients.

Magnetic resonance imaging (MRI) findings (**Figure 2**: panels a-e) of 6 patients included white matter changes (family 1: V-14, VI-10, and VI-12; family 3: V-1; family 4: III-4, and III-8), as well as abnormalities in the cerebellum, especially in the dentate nuclei (family 1: V-14, VI-10, VI-12; family 3: V-1). Atrophy

Table 1. Clinical, imaging, and molecular details of patients affected with L-2-hydroxyglutaric aciduria.

Family	1	1	1	1	1	1	1	2	3	3	4	4	4	4
Patient	IV-9	IV-10	V-14	V-16	VI-9[a]	VI-10	VI-12	V-5	V-1	V-2	III-3	III-4	III-6	III-8
Age[b] (y)	24	17	18	13	8	14	12	9	10	8	18	28	23	25
Gender	F	M	F	M	F	M	M	M	M	M	M	M	F	F
Urine L-2-HGA[c]	2167	1527	1384	1242	un	1846	1443	4142	1965	1670	un	5435	1385	1644
Occipitofrontal circumference (%)	norm	norm	55 cm (50th)	56.5 cm (98th)	un	53 cm (25th)	51 cm (5th)	56 cm	57 cm (98th)	56 cm (75th)	un	58 cm (>95th)	57 cm (75th)	57 cm
Seizures[d]	+	+	+ 3 y	+ 9 m, daily	un	+ 1 y	+ 4mo	+	+ 3 y	+ IF	un	+	+ IF	+ IF
Developmental delay	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Speech defect	+	+	un	+	+	+	+	un	severe	severe	un	+	+	+
Gait affected	+	+	un	+	+	+ ataxia	+ ataxia	+ ataxia	+ ataxia	un	un	+ ataxia	+	+
Mental retardation	+	+	+	un	un	+	un	un	un	un	un	un	un	Un
Brain MRI	nd	nd	AC, WM	WM, CA	nd	WM	WM	nd	WM, LK	WM, LK	nd	nd	nd	BT
Brain MRS	nd	nd	RCN	RCN	nd	ICN	RCN	nd	RCN	RCN	nd	nd	nd	nd
Brain CT	nd	nd	nd	LK	nd	nd	nd	nd	WM	nd	nd	nd	nd	nd
EEG	nd	nd	nd	nd	nd	DE	nd	nd	CI	nd	nd	nd	nd	nd
Other manifestations	CD, MD	CD, MD	CD, CS	CS, PR, QP, WM	BT	CS, CD,	CS,	MGH	CD, LD, AG, NY	CD	BT	ES, MC, RI	BT	un
Molecular findings	p. Arg 339Asp fsX351	p. Arg 339Asp fsX351	p. Arg 339Asp fsX351	p. Arg 339Asp fsX351	nd	p. Arg 339Asp fsX351	p. Arg 339Asp fsX351	p. Ser 440 Arg	p. Arg 339Asp fsX351	p. Arg 339Asp fsX351	nd	p. Gly 57Arg	p. Gly 57Arg	p. Gly 57Arg
Exons	8	8	8	8	nd	8	8	10	8	8	nd	2	2	2

AC: Atrophy of corpus callosum, AG: aggressive behavior, DE: diffuse encephalopathy, BT: brain tumor, EEG: electroencephalography, ES: extrapyramidal signs, CA: cerebellar atrophy, CI: increased cortical irritability, CD: cognitive dysfunction, CS: positive cerebellar signs, CT: computed tomography, F: female, ICN: increased choline and N-acetylaspartate peaks, IF: infrequent, L-2-HGA: L-2-hydroxyglutaric acid, LD: lordosis, LK: leukodystrophy, M: male, MC: macrocephaly, MD: motor delay, MGH: mild global hypotonia, MRI: magnetic resonance imaging, MRS: magnetic resonance spectroscopy, nd: not done, norm: normal, NY: nystagmus, PR: psychomotor retardation, QP: quadriparesis, RCN: reduced choline and N-acetylaspartate peaks, RI: recurrent cerebral infarction, WM: abnormal white matter changes, un: unknown.

[a]Died of brain tumor soon after diagnosis. [b]Describes the age when listed clinical details were obtained. [c]Values are in mmol/mol creatinine. [d]The age of onset of seizures and frequency of occurrence is listed when available. For patients VI-9 (family 1), III-3 (family 4), and III-6 (family 4), information about their brain tumors came from the clinical information of affected family members.

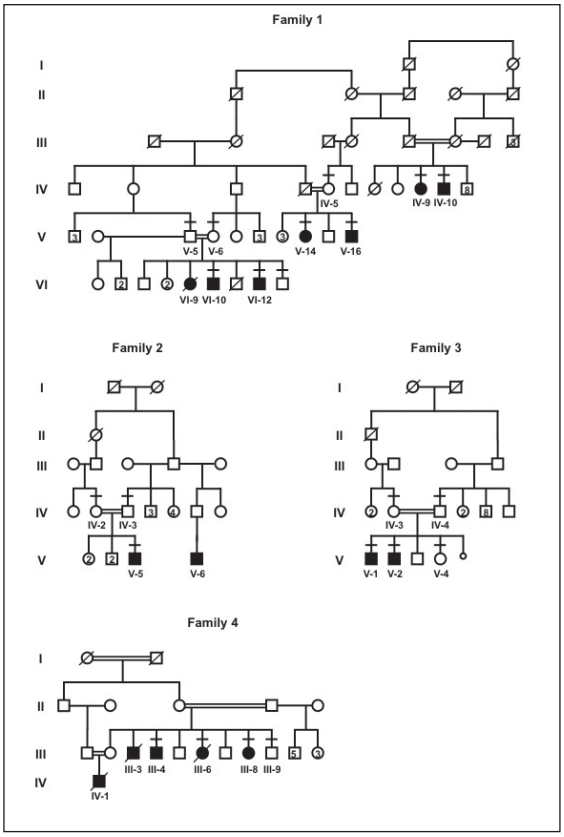

Figure 1. Family pedigrees of the L-2-hydroxyglutaric aciduria patients. Patients and unaffected family members whose DNA was sequenced are marked with horizontal lines above their symbols. Double lines indicate consanguineous marriage.

of corpus callosum was found in 1 patient (family 1: V-14). Corpus callosum was spared in the majority of these patients as of the thalami. In 1 patient, hyperintense lesion was located on the thoracocervical spinal cord. Computed tomography (CT) of brain (**Figure 2**), which was available for 2 patients, showed white matter changes (family 1, V-16, and family 4, III-8). Magnetic resonance spectroscopy (MRS) which was performed on 5 patients, showed reduced choline and/or N-acetylaspartate peaks in 4 patients, and increased choline and N-acetylaspartate peaks in 1 patient.

Mutation findings

Sequencing of the L2HGDH gene identified homozygous mutations in all patients from the four families. Their parents who were tested were heterozygous for these mutations (**Figure 3**). A deletion of single nucleotide, c.1015delA in exon 8, was found in individuals from families 1 and 3. This frame-shift mutation results in the p.Arg339AspfsX351 change in the protein. The missense c.1319C>A mutation in exon 10, which encodes for the p.Ser440Tyr substitution was found in family 2. In family 4, the missense c.169G>A mutation in exon 2 encoding for the p.Gly57Arg change was identified. The patients from this family also exhibited homozygosity of the c.159T, synonymous single-nucleotide polymorphism (SNP) variant (c.159C/T, rs2297995) (**Figure 3**).

DISCUSSION

This study was carried out to examine the spectrum of clinical manifestation of L-2-hydroxyglutaric aciduria and mutation analysis of the L2HGDH gene in Arab patients. Although biochemical features of L-2-hydroxy glutaric aciduria manifested in all patients, variable expression of associated phenotypes was seen in Arab patients.

The exact incidence of this disease in Arab patients is unknown as only few case reports have been described in the published reports. The typical age of presentation is infancy or early childhood, but patients with milder phenotypes may remain undetected until adulthood. The clinical presentation of these patients is specific to neurological system with developmental delays, motor dysfunction especially ataxia, convulsions/epilepsy, language regression, and mental retardation. 15 These features are constantly found in patients in the present study. The most consistent clinical findings in this study were developmental delays, gait disturbances (6 patients with ataxia), variable intensity of seizures, and language regression (speech defects). Severe speech abnormalities were seen in 3 patients recurrent cerebral infarctions in 1 patient.

While seizures manifest in most of these patients, the age of onset and frequency is variable. In the present study, the earliest seizures were noticed in a patient at the age of 4 months while in 2 patients from family 1 and family 3, seizures began at the age of 3 years. Tendency to develop brain tumors in L-2-HGA has been described in the published reports, which include medulloblastoma, primitive neuroectodermal tumor, glioblastome multiforme, and fibrillary astrocytoma. The exact cause of brain tumor in these patients remains unknown.[5] In the present study, 4 patients from 2 families were identified with brain tumors. Family 4, in particular, had a higher frequency of brain tumor occurrence, which was found in 3 of the 5 affected patients from this family. Unfortunately, the imaging studies to classify the brain tumors were not available, as these patients died before interviews.

In a recent study of 200 L-2-hydroxyglutaric aciduria patients, macrocephaly was described in 48% of the patients.3 In the present study, the OFC was >95% in 4

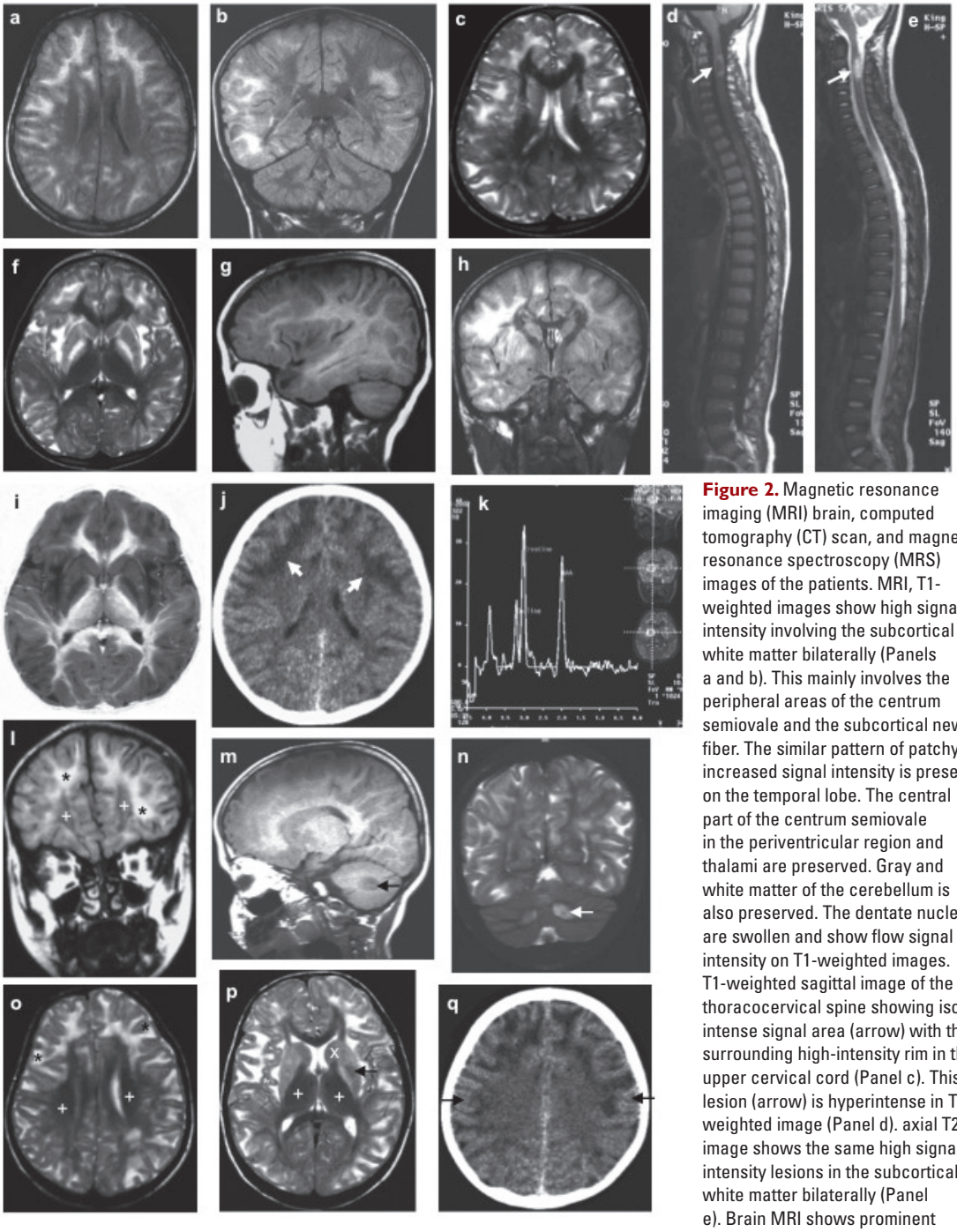

Figure 2. Magnetic resonance imaging (MRI) brain, computed tomography (CT) scan, and magnetic resonance spectroscopy (MRS) images of the patients. MRI, T1-weighted images show high signal intensity involving the subcortical white matter bilaterally (Panels a and b). This mainly involves the peripheral areas of the centrum semiovale and the subcortical new fiber. The similar pattern of patchy increased signal intensity is present on the temporal lobe. The central part of the centrum semiovale in the periventricular region and thalami are preserved. Gray and white matter of the cerebellum is also preserved. The dentate nuclei are swollen and show flow signal intensity on T1-weighted images. T1-weighted sagittal image of the thoracocervical spine showing iso-intense signal area (arrow) with the surrounding high-intensity rim in the upper cervical cord (Panel c). This lesion (arrow) is hyperintense in T2-weighted image (Panel d). axial T2 image shows the same high signal intensity lesions in the subcortical white matter bilaterally (Panel e). Brain MRI shows prominent dentate nuclei and globi palladi with abnormally increased signal intensity, sparing of the thalami, and extensive leukodystrophy with peripheral predominance (Panels f-i). CT of the brain shows extensive white matter, low-attenuation areas involving both cerebral hemispheres, which is suggestive of leukodystrophy (Panel j). This involves the frontal pole more than the occipital and temporal lobes. MRS shows prominent reduction of the choline peak with mildly decreased N-acetylaspartate peak (Panel k). Coronal MRI of T1-weighted image shows bilateral subcortical white matter changes (*) with sparing of periventricular white matter (+) (Panel l). Sagittal T1-weighted image of brain shows cerebellar dentate nucleus lesion (arrow) (Panel m). Coronal T2-weighted image of brain shows a bilateral dentate nuclear lesion, which is prominent on the left side (arrow) (Panel n). Axial, T2-weighted image of brain shows deep periventricular white matter sparing (+) with the involvement of subcortical white matter (*) (Panel o). The axial T2-weighted image of brain shows sparing of thalami (+) with the involvement of caudate (x) and lentiform nuclei (arrow) with subcortical white matter changes (*) (Panel p). CT of brain axial image shows subcortical white matter changes bilaterally (arrows) (P and q).

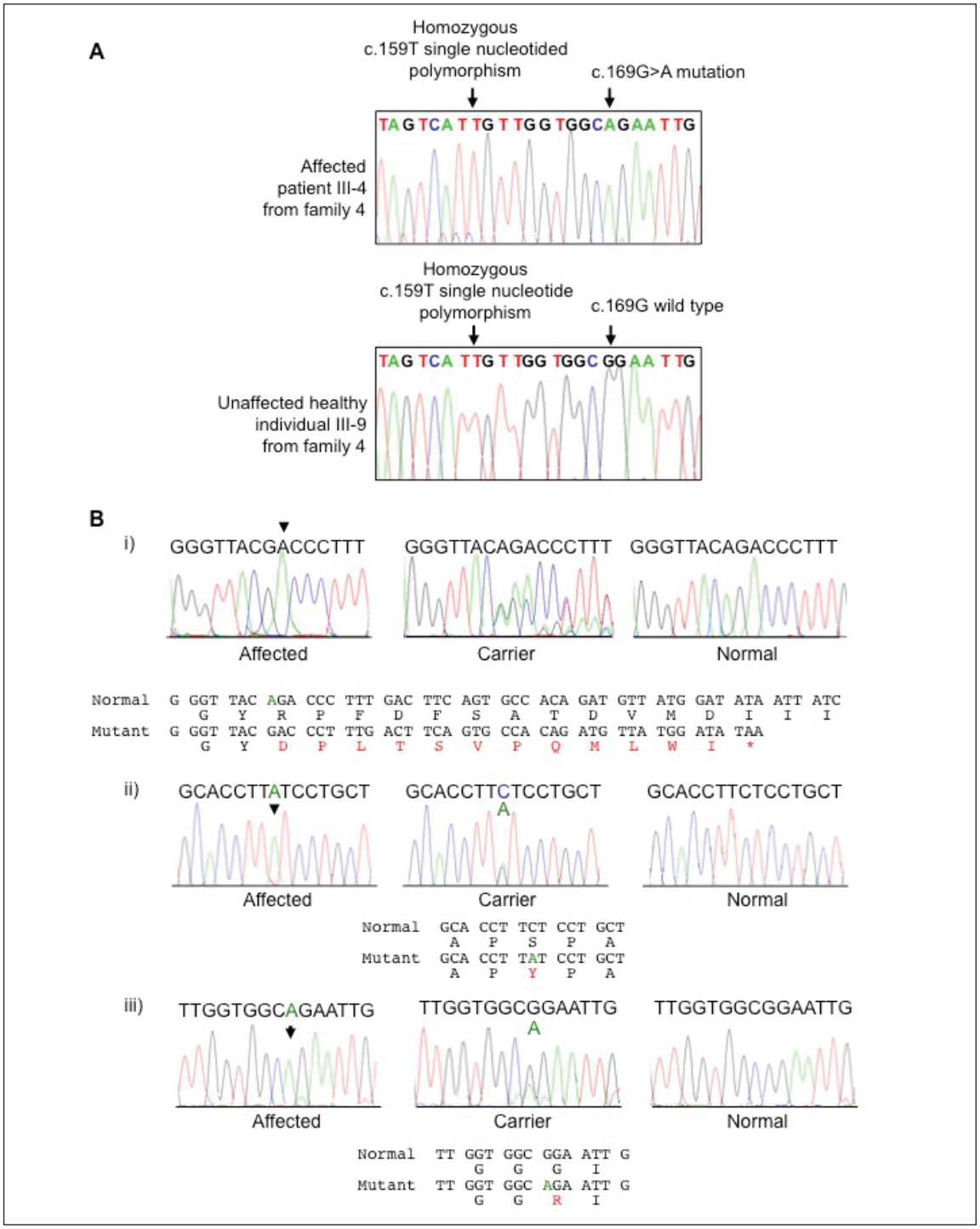

Figure 3. (A) Chromatogram of a patient with the c.169G>A mutation. The upper panel depicts the homozygous c.169G>A mutation and the homozygous c.159T single-nucleotide polymorphism (SNP) in the L2HGDH gene of a patient from family 4. The lower panel shows the wild-type homozygous c.169G and the homozygous c.159T SNP in the L2HGDH gene of an unaffected individual from family 4. (B) Sequence analyses of mutations in the L2HGDH gene. Homozygous mutations in L2H aciduria patients are shown in sequence chromatograms on the left (arrowheads), heterozygous (carrier) sequence chromatograms on the middle, and normal sequences from control subjects on the right panels. The upper panel (i) depicts the p.Arg339AspfsX351 mutation from family 1 and family 3, the middle panel (ii) depicts the p.Ser440Tyr mutation from family 2, and the lower panel (iii) shows the p.Gly57Arg mutation from family 4.

patients (28.5%). Neuroimaging findings were specific for this condition including white matter changes, involvement of basal ganglia, and cerebellum with sparing of corpus callosum and brainstem. Subcortical white matter was extensively affected with detection of abnormal signals in the dentate nucleus and atrophy of cerebellum. Brain MRI of 6 patients and brain CT of 2 patients showed a variety of abnormalities, such as white matter changes, atrophy of the cerebellum, and increased signal intensities in various regions like dentate nucleus. Corpus collosum is typically spared in this disease, but 1 of our patients had atrophy of corpus callosum similar to the case reported by Seijo-Martínez et al.[13] MRS findings of altered choline and N-acetylaspartate peaks, which were seen in 5 patients in the present study, have been described in other patients.[4,14] In the present study (with the exception of 2 uncommon imaging signs: thoracocervical spine lesion and corpus callosum atrophy), brain MRI is highly suggestive and pathognomonic of L-2-hydroxyglutaric aciduria. Our study indicates variability of clinical presentations in Arab patients. Similar heterogeneity of disease manifestation has been described in patients from other ethnicities.[3,4,9]

Sequencing of the L2HGDH gene has revealed 2 missense (c.1319C>A and c.169G>A) and a frameshift (c.1015delA) mutations in patients from 4 Arab families. This is the first report of the recurrent c.169G>A mutation in Arab patients. In glutaric aciduria patients, mutations shared by different populations are rare. Only mutations encoding for the Pro302Leu have been described in different populations.[6-8,10] In a recent study, the c.169G>A mutation (p.Gly57Arg) that was originally found in a compound heterozygous state in a Portuguese female was discovered in a homozygous state in an Italian male patient.[7,10] Haplotyping showed that the Portuguese and Italian patients shared the mutation-carrying chromosome with identical SNP haplotypes, thus pointing to a common origin of the mutation in these 2 populations.[10] The Italian and Portuguese patients carried the c.159C allele (c.159T/C SNP, which is located 10 nucleotide upstream of the pathogenic c.169G>A mutation.[9] The finding of the homozygous c.159T allele associated with the c.169G>A mutation in Arab patients points to an independent origin of the c.169G>A mutation in Arab population. The data also suggest that the c.169G may be a mutation hotspot in the L2HGDH gene and that this mutation may also exist in populations of other ethnicities.

In the present study, the heterogeneity of manifestations of clinical and radiological features is similar, and there do not seem to be any distinct features among the 3 different mutations. These findings corroborate data in the published studies, as there is no correlation between known mutations and phenotype severity.[15] The detailed clinical description and mutation analysis in this study is useful for the diagnosis of L-2-hydroxyglutaric aciduria patients of Arab and other ethnic origins.

Acknowledgment

This manuscript was supported by the College of Medicine Research Center, Deanship of Scientific Research, King Saud University.

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