

Case report.

Progressive Early Onset Leukodystrophy Related to Biallelic Variants in the KARS Gene: The First Case Described in Latin America

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Abstract: The KARS gene encodes the aminoacyl-tRNA synthetase (aaRS) which activates and joins the lysin with its corresponding transfer RNA (tRNA), through the ATP-dependent aminoacylation of the amino acid. The KARS gene mutations have been linked to diverse neurologic phenotypes such as: neurosensorial hearing loss, leukodystrophy, microcephaly, developmental delay or regression, peripheral neuropathy, cardiomyopathy, impairment of the mitochondrial respiratory chain, hyperlactatemia, among others. This article presents the case of a Colombian pediatric patient with two pathological missense variants in a compound heterozygous state in the KARS gene.

Keywords: KARS gene; aminoacylation; leucodystrophy; epilepsy; hearing loss developmental delay; whole exome sequencing

1. Introduction

Aminoacyl t-RNA synthetases (aaRSs) are 20 enzymes fundamental in the translation of messenger RNA (mRNA) to proteins in eukaryotic cells. Each aaRS mediates the initial adenylation of the amino acid, and the subsequent formation of an ester bond between aminoacyl-AMP and its specific t-RNA; this process is denominated aminoacylation and it could take place in the cytoplasm, mitochondria, or in both compartments in the cell [1–6].

The Lysyl-tRNA synthetase (LysRS) which is a bifunctional aaRSs, is encoded by the KARS gene [OMIM # 601421]; this gene is located on the chromosome 16q23.1, and encompasses 15 exons; it is translated into the two Lysyl-tRNA synthetase protein (LysRS) isoforms, through alternative splicing. These forms of the protein differ mainly in their amino terminal end, which defines their final location in the cytoplasm or in the mitochondria [2,7,8].

Due to the central role of aaRSs in decoding the genetic code, the alteration of their function leads to various diseases with an autosomal recessive pattern of inheritance, with a broad neurological phenotype, with or without systemic involvement.

Pathogenic variants in the KARS gene have been associated with autosomal recessive non-syndromic sensorineural hearing loss, congenital visual impairment, progressive microcephaly, leukodystrophy,

some variants of Charcot-Marie-Tooth disease, severe cardiomyopathy related to mild to severe myopathy, developmental delay, cognitive impairment, epilepsy, among others [2,4,9,10].

2. Materials and Methods

Before the initiation of the study, the acceptance from the Academic Research Ethics Committee of the Clínica Universidad de la Sabana was obtained, and the informed consent was signed by the patient's parents under the guidelines of the Declaration of Helsinki; after this, the review of clinical record and physical examination of the patient were performed.

Whole exome sequencing (WES) of patient's DNA was requested, including the analysis of approximately 23,000 genes, the exon capture with the Nextera Exome Capture® System, followed by NGS sequencing by Illumina® platform; the median depth of coverage was 66 readings, with 92% of all coding exons included.

The literature search was performed in databases such as Clinicalkey, PubMed, Access Medicine, OMIM to proceed with the comparison of the case with the available information.

3. Results

3.1. Clinical case

A male patient from Colombia was first evaluated by pediatric neurology at six months of age, because of a history of a global development delay and absence of responses to auditory stimuli. He is the first child of non-consanguineous parents, with an unremarkable gestation, born at term by assisted vaginal delivery, without any other complication (Figure 1). In the family history, the father reported a paternal aunt with seizures and motor impairment of unknown cause, with apparently no auditive nor visual dysfunction, who died during adolescence.

At the age of twelve months, the patient suffered a sudden neurological regression associated with an intercurrent febrile event of unclear etiology; it comprised the loss of language and all the motor milestones, even the head support. Also, visual deterioration was evident due to the absence of fixation and object tracking by the child.

During the periodical follow-up, the neurological decline of the patient included a progressive paresis of the four limbs, and impaired swallowing, making the use of a gastrostomy necessary. At 6 years old, the patient presented focal motor-tonic seizures, which were treated with levetiracetam and valproic acid with complete control of them.

On physical examination, the patient does not have eye-gaze fixation nor following; there is no answer to auditory stimuli nor follow of commands. the child does not emit any verbal language, and has a spastic quadriplegia, without cephalic control or manipulation of objects; fasciculations in the abdomen were also evident.

3.1.1. Laboratory studies

Studies of lactate, pyruvate, quantitative amino acids by high-performance chromatography in blood and urine, enzymatic activity of hexosaminidase A, ammonia, and serum long- chain fatty acids, were found at normal values.

3.1.2. Electrophysiological studies

Auditory evoked potentials at 10 months of life shown a severe sensorineural hearing loss; on the other hand, the visual evoked potentials taken at one year of age, presented prolonged latencies of N75-P100-N145 waves, suggestive of demyelination of the retino-genico-calcarin pathway.

3.1.3. Images

The Magnetic resonance imaging of the brain (MRI) performed at 10, 16 and 96 months-old, showed a marked progressive cortical atrophy associated to a diffuse ventriculomegaly; also, a enhanced signal in the T2-weighted and FLAIR images in the splenium of the corpus callosum, thalamus and the white matter of the temporal, parietal, occipital lobes and the cerebellum were evident. The spectroscopy revealed a decreased N-acetyl-aspartate peak, in contrast to a high peak of lactate (see image 1-3).

3.1.3. Genetic studies

In the absence of an etiology of the patient's condition, a WES was performed where two variants in the KARS gene were identified; the variant c.1514G > A (p.Arg505His) classified as pathogenic (rs778748895) previously reported in the Literature (4,11,12). The variant c.371G > A (p.Ala526Val), which has been classified as probably pathogenic, has not been previously reported in the literature. These variants are in trans configuration which confirms a state of compound heterozygosity.

Genes search:

1. Clinical VAR: National Center for Biotechnology Information. ClinVar;[VCV000694746.2], <https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000694746.2> (accessed Feb. 12, 2020).
2. Prediction in silico: Varsome Clinical. A Clinical-grade Platform for interpretation of NGS Data, [https://varsome.com/variant/hg19/NM_001130089.1\(KARS\)%3AA526V](https://varsome.com/variant/hg19/NM_001130089.1(KARS)%3AA526V) (accessed Apr. 10, 2020).

3.2. Figures, Tables and Schemes

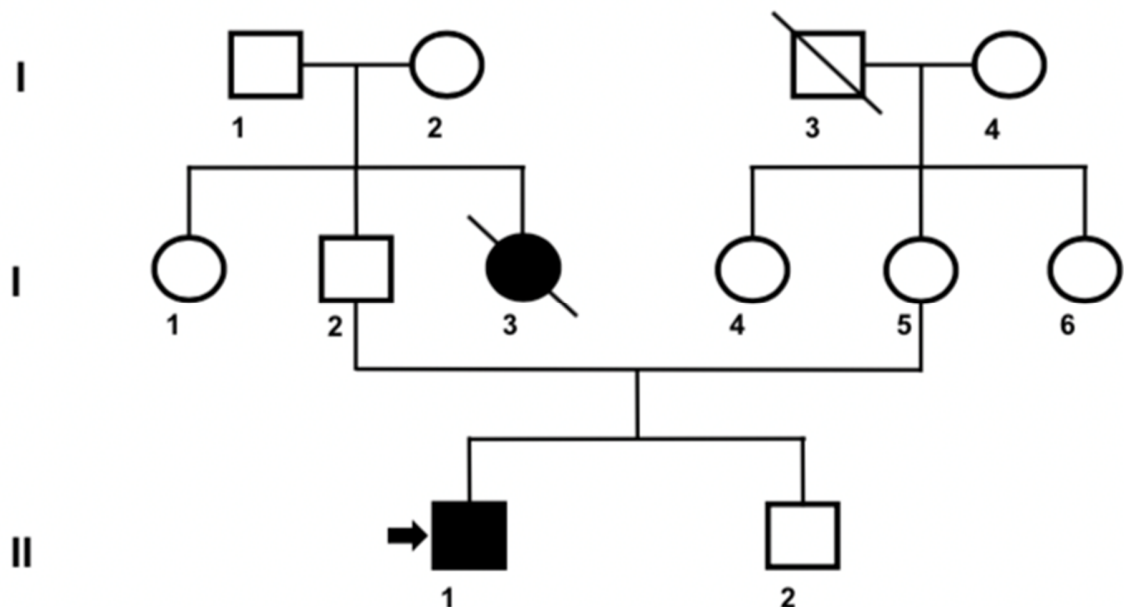


Figure 1. Genealogy of three generations: Grandparents, Parents and Brothers. The death of maternal grandfather (GI: # 3) resulting from gastric cancer is observed in the first generation (GI: Grandparents). In the second generation (GII: parents, uncles and aunts), the father's younger sister (GII: # 3) presented a neurological phenotype without a clear etiology and death during adolescence (GII: # 1).

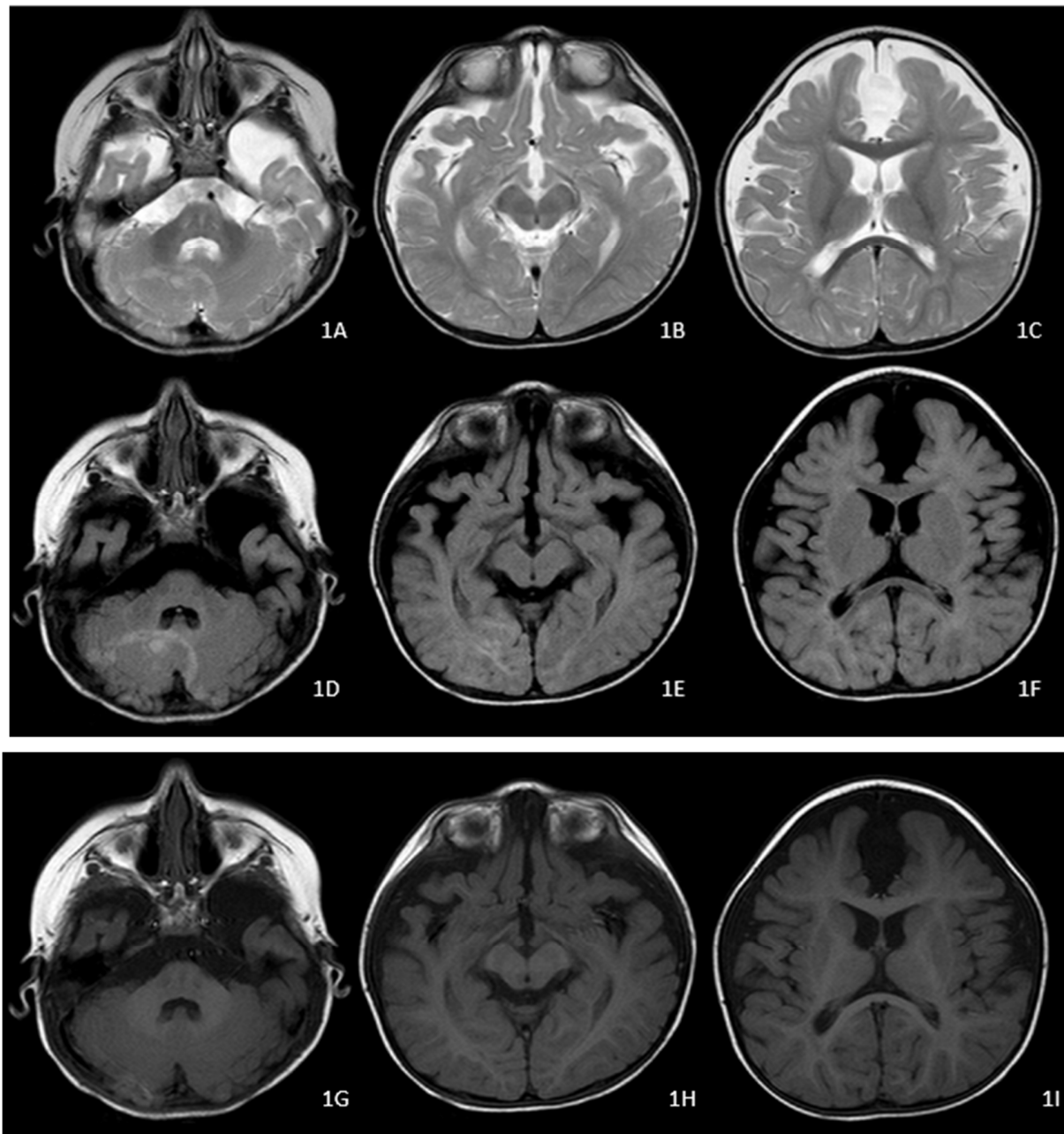
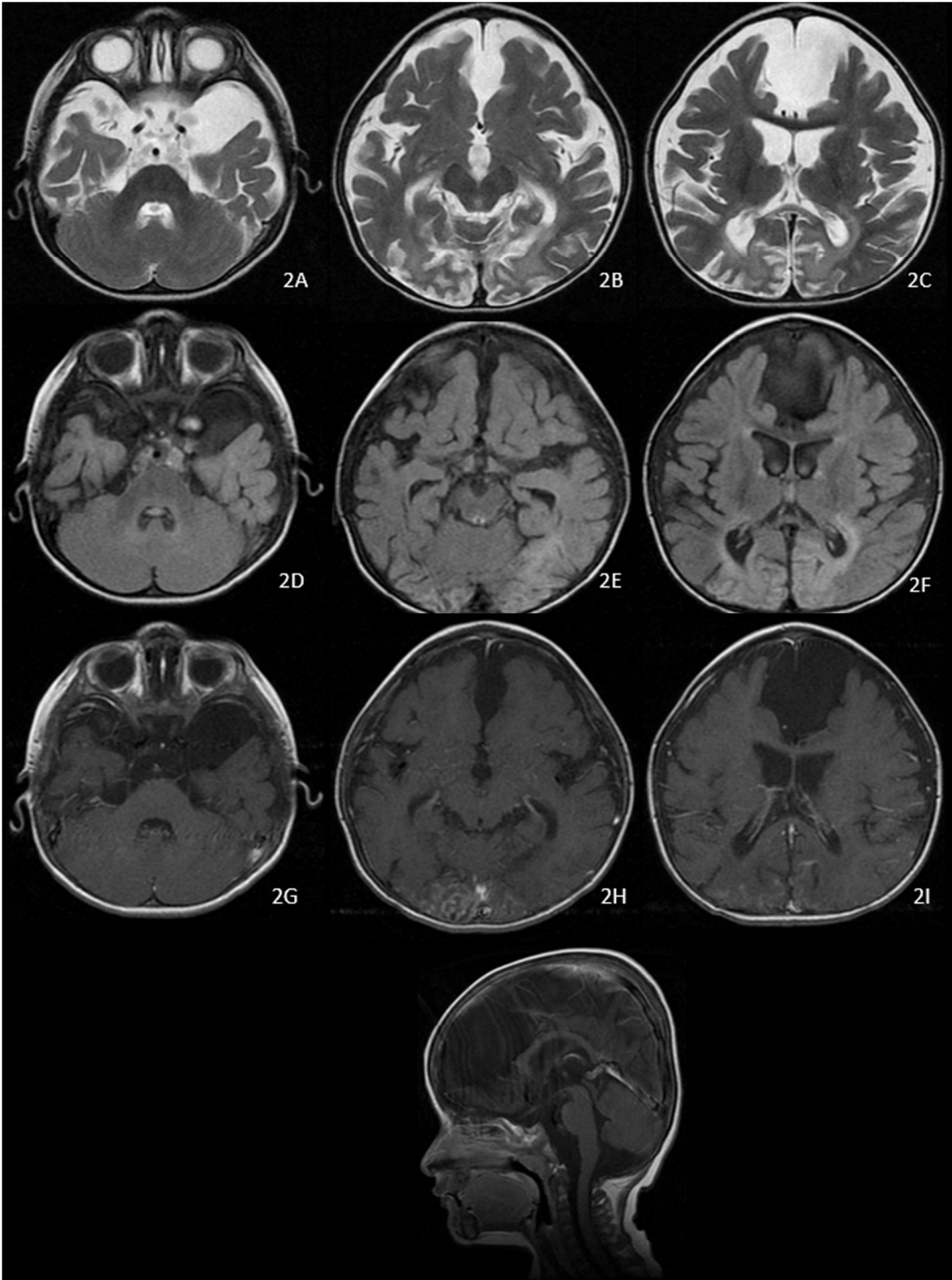


Image 1. Brain Magnetic Resonance imaging (10 months-old): In the fluid-attenuated inversion recovery (FLAIR) sequence, at the level of the middle cerebellar peduncles in the pons, lateral to medial lemniscus, there is a linear hyperintensity area, which looks hypointense in the T1 spin echo image. In the T2-weighted, and FLAIR images, a prominent subarachnoid space is evident, with the presence of an interhemispheric and a left temporal arachnoid cyst. A mild delay of the myelination is observed at the corona radiata, with a thinner splenium of the corpus callosum. There are no significant changes in the diffusion-weighted imaging (DWI) nor in the apparent diffusion coefficient (ADC).



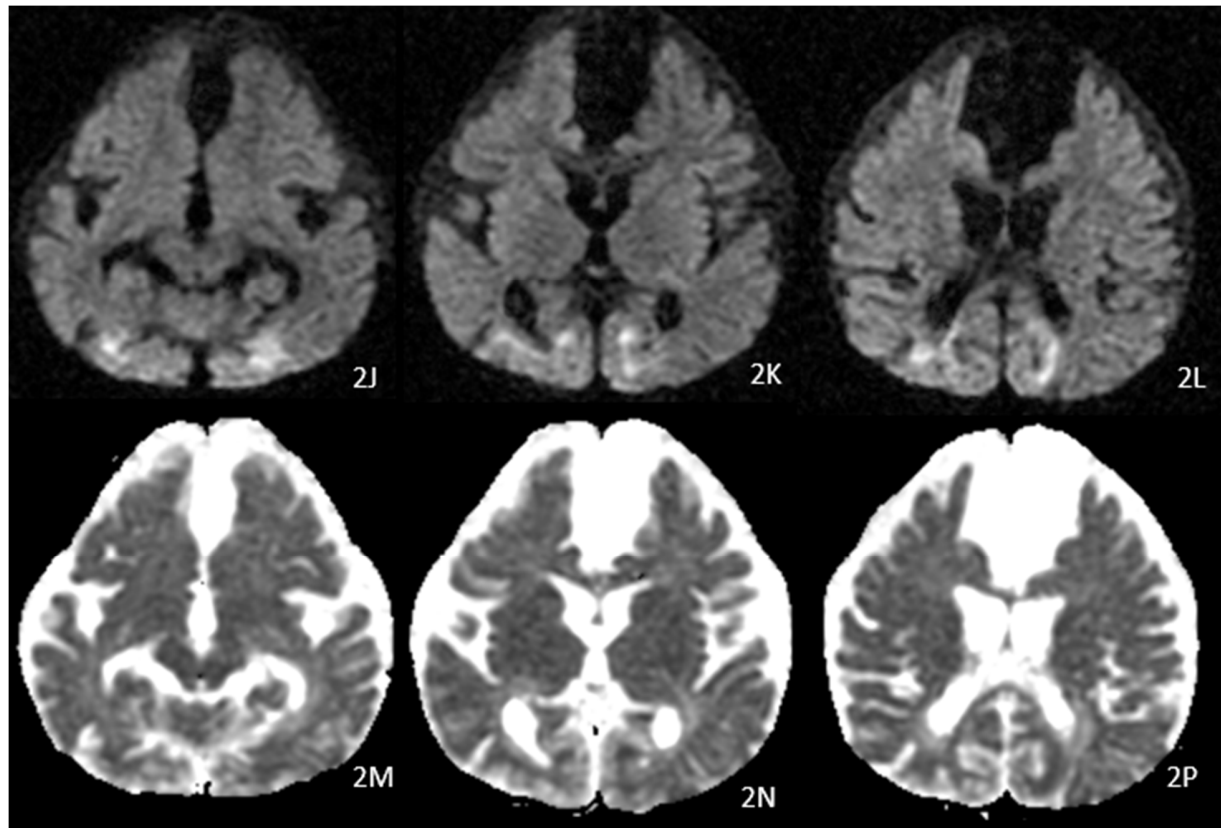
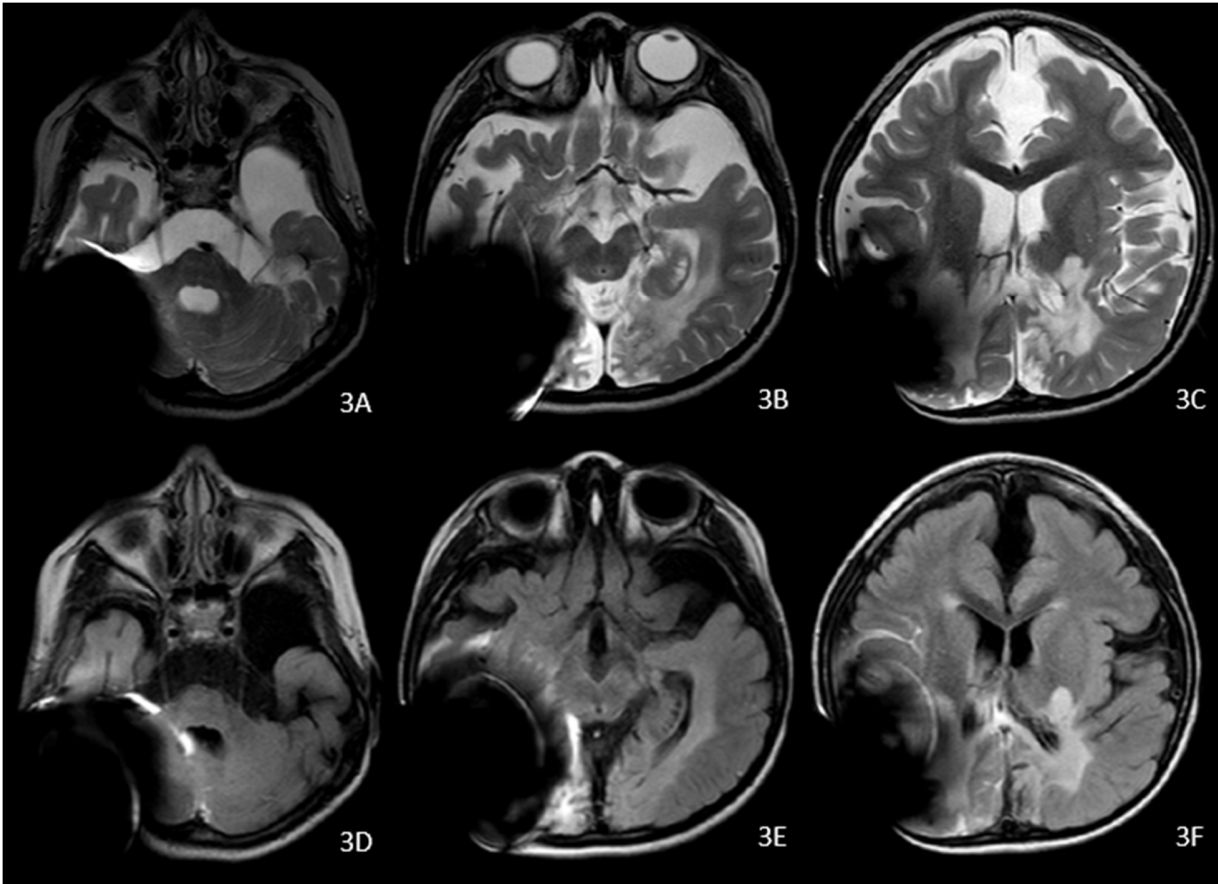
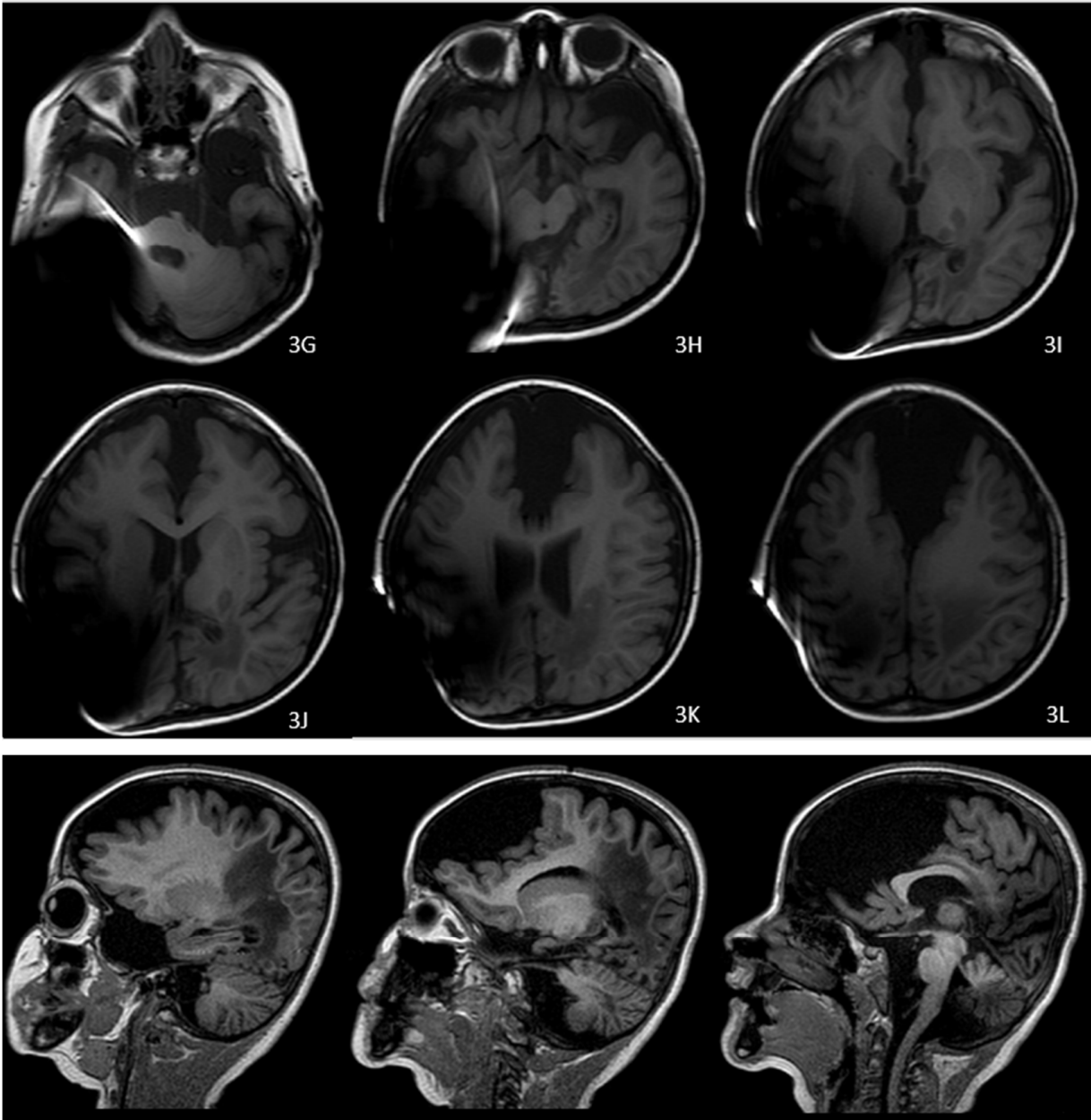
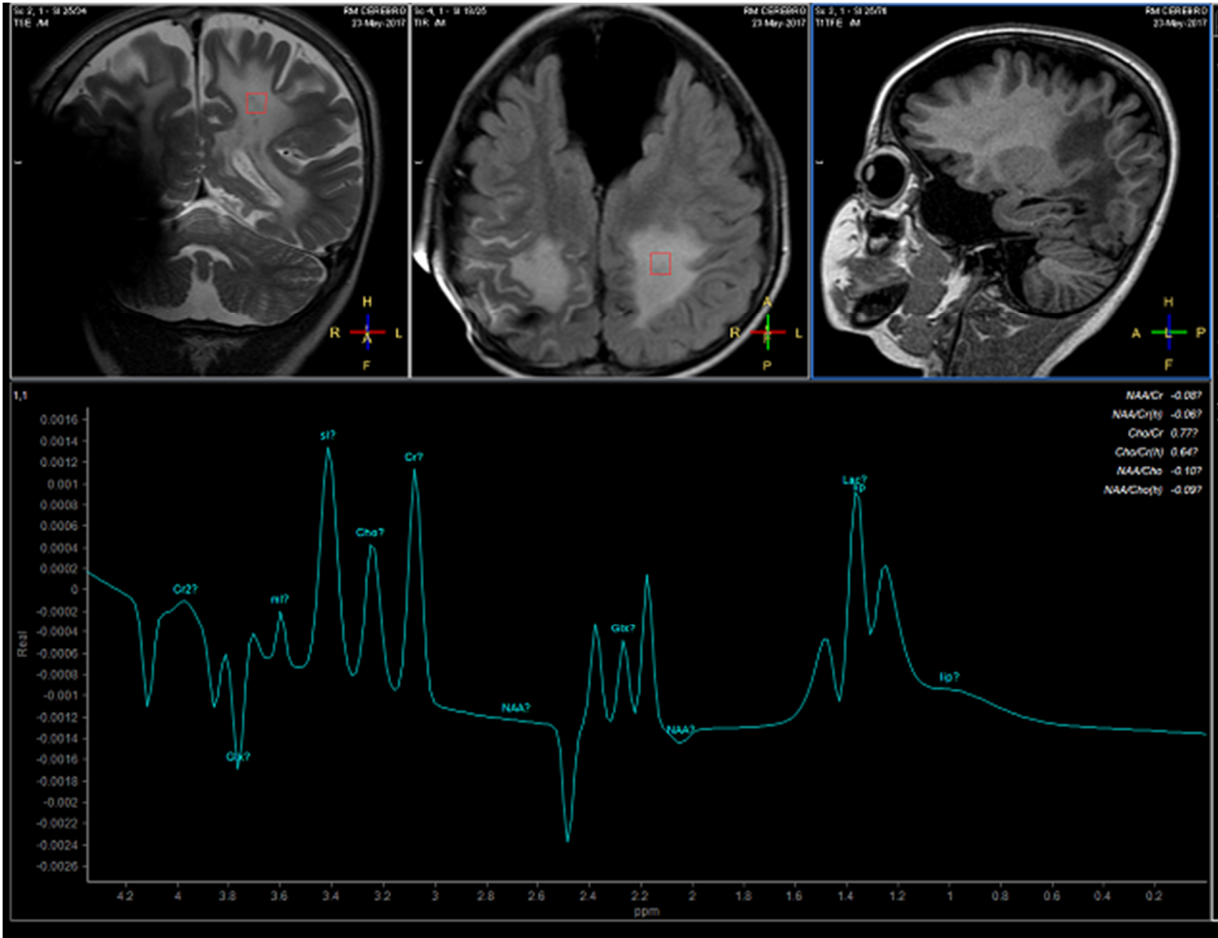


Image 2. Brain Magnetic Resonance imaging (1 years, 4 months-old): In the T1, T2-weighted and FLAIR images, a marked generalized cortical atrophy is evident. In the T2-weighted and FLAIR images, a diffuse cortical atrophy is evident. Increased signal areas are observed in the perirhinal area, corona radiata, and the white matter of the parietal and occipital lobes predominantly; also, gliotic changes are present bilaterally in the occipital poles. A compensatory ventriculomegaly is evident. T1-weighted sagittal and axial images: There is no enhancement with gadolinium. In DWI areas of high signal in the occipital white matter are observed; there are not significant changes in the ADC.







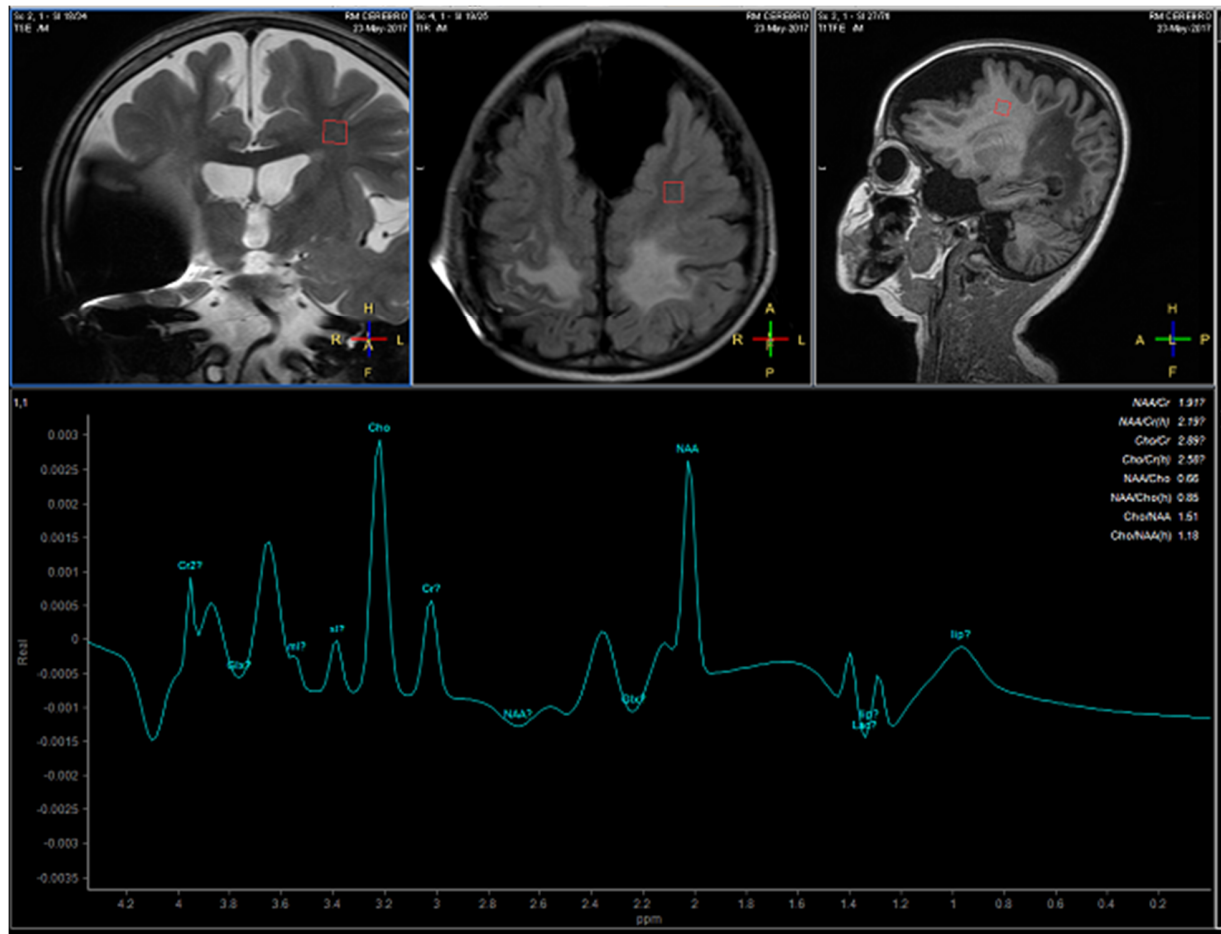


Image 3. Brain Magnetic Resonance imaging (3 years, 8 months-old): In the T2-weighted and FLAIR images, there is an increased signal compromising the right head of the caudate nucleus, the thalamus, the posterior arm for the internal capsule, the splenium of the corpus callosum, the posterior third of the cingulate gyrus, the temporo-parieto-occipital and cerebellar white matter bilaterally. In the T1-weighted image, the previously described areas are hypointense. In the spectroscopy, in the voxel located in the parietal white matter, the N-acetyl aspartate peak is importantly diminished, with a high lactate peak.

4. Discussion

A variable clinical presentation of the disease has been associated with the KARS gene mutations; all those disorders, have been linked to an autosomal recessive inheritance pattern mechanism [12,13]. Among the most affected organs, are those with high energy demand such as the brain, heart, skeletal muscle and kidneys, probably secondary to the mitochondrial dysfunction with a secondary impaired oxidative phosphorylation [13].

The wide spectrum of neurological phenotypes comprises the peripheral neuropathies with a variable severity of disease, microcephaly, developmental delay, cognitive decline, epilepsy, ataxia, hypotonia, spasticity, hemiplegia, quadriplegia, abnormal movements such as dystonia and chorea, leukoencephalopathy, cerebral white matter, brainstem and spinal cord calcification, visual impairment and sensorineural hearing loss [2,4,9,11–16].

The leukodystrophies comprise the genetic diseases that affect predominantly the white matter, with damage of the glial cells and myelin sheaths [17]. Gliosis and demyelination, without significant compromise of the cerebral cortex has been described in the in brain pathologic analysis two patients with

KARS mutations [15]. The pathologic and image findings described in our patient and in the literature, support the classification of the white matter injury associated to LysRS mutations as a primary leukodystrophy (LD) [16,18].

Imaging studies represent a gateway to the diagnostic approach, with a wide repertory of findings been described in KARS LD. These include the progressive thinning with symmetric hyperintensity of the cerebral white matter in the T2 and FLAIR images, including or not the U fibers [2,12,13].

Also cortical atrophy, gliotic changes, hypomyelination, demyelination, ventriculomegaly, increased signal in the internal capsule, corticospinal tracts, thalamus, substantia nigra, cerebellar peduncles, and corpus callosum dysgenesis have been described [2,11–13]. In the spectroscopy, a reduced N-acetyl-aspartate peak, with an elevated or diminished lactate peaks in the white matter have been found [11,12,16,18].

A remarkable sign that has been described in some of the patients with KARS mutation, is the calcification of the cerebral white matter, basal ganglia, internal capsules, cerebellar nuclei, brainstem and spinal cord [11,12,14].

Our patient presents a severe neurological phenotype like what has been described previously in the literature, with a marked clinical deterioration over time. The brain images showed an extensive and rapidly progressive cortical atrophy, loss of the white matter, corpus callosum dysgenesis, calcifications, and gliotic lesion of the white and gray matter in the occipital lobes.

For undiagnosed patients with white matter disorders of suspected genetic etiology, the WES has emerged as a diagnostic test that allows to determine a definitive etiology in approximately 70% of the patients; this figure could be increased to an 80% with the use of genome sequencing [19,20]. In our case, the use of WES in trio allowed the achievement of a definitive diagnosis for the patient.

KARS mutations have been linked to non-syndromic hearing impairment, without other neurological relevant symptoms. KARS expression has been specially demonstrated in animal models at the Organ of Corti, specifically in the spiral ligament of the cochlea, inner and outer hair cells, tectorial membrane, supportive Deiter's cells, basilar membrane, spiral ligament, spiral limbus epithelium and inner sulcus cells [8].

The KARS mutations has been proposed as the cause of the secondary malfunction of the structures previously indicated in the inner ear, as they are in charge of the transduction of the mechanic stimuli to an electric signal; this has been proposed but still requires further studies [8]. In our patient this could explain the early hearing loss, which could also deteriorate in time, secondary to the white matter progressive damage.

The use of WAS in this patient resulted in the identification of two variants in the KARS gene; the first pathogenic variant was already reported in the literature, c.1514G > A (p.Arg505His). This variant has been identified in two cases of patients with sensorineural hearing loss and leukodystrophy [4,11,12].

This amino acid change occurs in a highly conserved position in various species, so the In Silico prediction concludes that it is deleterious. This variant is present in heterozygosity in one of 138,000 individuals in the world population and has been shown in vivo to affect enzyme activity leading to a decrease in lysine t-RNA aminoacylation and is therefore considered pathogenic.

The second variant c.371G > A (p.Ala526Val) has not been previously described in the medical literature nor associated with phenotypes related to KARS in humans. This change also occurs in a highly conserved residue, analyzed with 16 bioinformatic predictors that conclude that it is deleterious ([https://varsome.com/variant/hg19/NM_001130089.1\(KARS\)%3AA526V](https://varsome.com/variant/hg19/NM_001130089.1(KARS)%3AA526V)).

Both variants are located in the catalytic domain of the LysRS, which contains the site to activate the amino acid lysine and links it to the corresponding t-RNA through aminoacylation; the collective effect of these changes could reduce the amino-acylation of l-lysine t-RNA.

Mutations of the KARS gene represent a diagnostic challenge given the little literature available, in addition to a wide clinical phenotype, which could be an isolate symptom, such as the non-syndromic

hearing loss, or a mild to severe neurological disease [2,4,8,11,12,14,16,18]. In addition, identifying a KARS gene mutation as the etiology in a specific patient, could impact on the management, prognosis, and genetic counseling.

5. Conclusions

This is the first case of a patient with a KARS mutation associated to a severe neurological phenotype reported in Latin America. The pathogenic variants in the KARS gene are responsible for the LysRS deficiency that generates various alterations in neurological development and mitochondrial function, however, new phenotypes attributable to these variants continue to emerge today as a recognizable cause of rare diseases in the pediatric population.

Given the variety of clinical expression for mutations in KARS, our case shows that the biallelic pathogenic variants in this gene may be responsible for the severe clinical phenotype that is highlighted by hearing loss and severe early-onset leukodystrophy, which is probably the result of a reduced activity of LysRS.

It is important in children with cognitive and neurological impairment, with extensive compromise of the central nervous system white matter, to promote the use of WES and genome sequencing if the initial metabolic and enzymatic assays are negative. The availability of these diagnostic tools has significantly increased the likelihood of achieving a definitive diagnosis in most of the patients with a suspected genetic etiology of the disease.

Author Contributions: Conceptualization, I.A, S.V, M.C and C.P.; methodology, I.A, M.C, S.V and C.P; validation, A.V, J.R and L.C.; formal analysis, A.V and J.R.; investigation, I.A, S.V and M.C.; writing—original draft preparation, I.A, S.V and M.C.; writing—review and editing, A.V, J.R and L.C.; visualization, A.V, J.R and L.C.; supervision, A.V, J.R and L.C.; project administration, X.X. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: In this section you can acknowledge any support given which is not covered by the author contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

Conflicts of Interest: The authors declare no conflict of interest.

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