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Report of a progressive leukoencephalopathy with ovarian failure (LKENP) case with compound heterozygous genotype and a novel variant: *AARS2*:c.2358_2364+7dup

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Abstract

Leukoencephalopathies are a heterogeneous group of diseases in which many acquired and hereditary factors play a role in its etiopathogenesis. In recent years, Alanyl-tRNA synthetase 2 (*AARS2*), encoded by the nuclear genome, has been identified as the causative gene in a small number of patients. The *AARS2* gene is responsible for the "progressive leukoencephalopathy with ovarian failure (LKENP)" phenotype, an extremely rare syndrome characterized by progressive leukoencephalopathy and premature ovarian failure. In this case report; we describe the delayed diagnosis of LKENP by genetic analysis using a large gene panel, in a female who was followed up in different clinics for many years with clinical findings of early ovarian failure, amnesia, depression, young-onset dementia, early ovarian failure and leukoencephalopathy. As a result of genetic analysis of the patient using NGS-based targeted multigene panel testing, disease-related variants in the *AARS2* gene were found to be compound heterozygous. These; reported in the literature as NM_020745.4(*AARS2*):c.1709delG (p.Gly570AlafsTer21) and novel NM_020745.4(*AARS2*):c.2358_2364+7dupCCAGCAGGTCAGCA variants. When the clinical and radiological findings observed in our case were evaluated together, LKENP was considered in the preliminary diagnosis among all adult-onset leukoencephalopathy types. In this rare hereditary type of leukoencephalopathy, molecular genetic tests was important in elucidating etiopathogenesis.

Keywords: Leukoencephalopathy; next-generation sequencing; *AARS2*; multigene panel; novel mutation

INTRODUCTION

Leukoencephalopathies are a group of neurological diseases characterized by progressive degeneration of cerebral white matter.[1] Among hereditary causes, progressive leukoencephalopathy (LKENP) is a rare type presenting with progressive symptoms of cognitive decline and gait dysfunction that manifest at adulthood. LKENP is caused by mutations of the *AARS2* gene.[2] LKENP is a very rare subgroup of leukoencephalopathy, which has been reported in only 21 cases. Neurological findings accompanied by ataxia and spasticity are more severe in patients with early-onset disease. Premature ovarian failure is observed in females affected by this disease.[3]

In this report, we describe the process of making the diagnosis of LKENP by finding the causal *AARS2* gene with genetic analysis of a female patient who applied to the doctor with the complaint of forgetfulness.

CASE REPORT

A 47-year-old female patient applied to a psychiatry clinic 8 years ago with the complaint

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of forgetfulness and was diagnosed with depression. Her medical history revealed that she had secondary amenorrhea developing after a cesarean section at the age of 17 and hypothyroidism. Despite the depression treatments given to the patient, her depressive mood, memory and concentration problems continued to increase and she was diagnosed with young-onset dementia. Brain MRI was performed to elucidate the etiopathogenesis of the disease. When signs of white matter involvement were detected in the patient on MRI examination, she was referred to the neurology department. Neurological examination of the patient revealed that she was oriented and cooperative. Cranial nerve examinations were normal. The motor examination revealed mild paralysis in the lower extremity. Sensory and cerebellar examinations were within normal limits. The deep tendon reflexes were increased and the both Babinski sign was positive. The standardized mini-mental state examination score was 20 points. The laboratory investigations were within normal ranges. Further investigations to search for hereditary leukoencephalopathies were also performed (very long-chain fatty acids, galactocerebrosidase, arylsulfatase A, plasma amino acid profile) which yielded insignificant results. Cranial MRI showed frontoparietal predominant, symmetric, confluent T2-hyperintense white matter signal abnormalities suggesting leukoencephalopathy (Figure 1). Based on neuroimaging findings and early ovarian failure, leukoencephalopathy with vanishing white matter was considered in the preliminary diagnosis and performing genetic testing was decided. The genes responsible for this phenotype, *EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5*, were found to be normal. The patient's parents were second-degree relatives (nephews), and no history of a similar clinic was present in any of the members of the pedigree. The patient's mental decline progressed rapidly in the next 6 months and epilepsy developed. Epilepsy treatment (levetiracetam 250mg bd) was started. MRI was repeated and a marked increase in abnormal findings was observed over a 9-month period (Figure 1). Since the clinical picture of the patient deteriorated and a definitive diagnosis could not be made, it was planned to investigate the etiopathogenesis of the disease with an NGS-based targeted multigene panel.

METHODS

The genetic analysis was performed on the Nextseq Platform (Illumina, USA) using TruSight_One_Expanded kit. Genes associated with the clinical findings of the patient were examined. Raw data obtained by NGS were analyzed in a web-based bioinformatics program (https://www.sophiagenetics.com/home.html) and according to the reference genome (GRCh37(h19)). Variations detected by 30X read depth per allele were evaluated. Single base mutations and small deletions/duplications in the coding regions (exons) and exon-intron boundaries (20 base pairs) were investigated. The evaluation of the variants was made according to the ACMG criteria, taking into account the available information in the relevant databases.

Variant interpretation

Disease-associated variants in the *AARS2* gene were found as compound heterozygous (Figure 2). Neither variant has been previously reported in population databases. Of these variants; NM_020745.4(*AARS2*):c.1709delG (p.Gly570AlafsTer21) is a pathogenic variant that causes a frameshift mutation in the 12th exon of the *AARS2* gene and is previously known in the literature.[3] The other one NM_020745.4(*AARS2*): c.2358_2364+7dupCCAGCAGGTCAGCA, is a likely pathogenic novel variant in which 14 base pair size insertion is observed in the 17th intron of the *AARS2* gene. PM1+PM2+PP3+PP4 scores were applied in the evaluation of this variant (Table 1). The variant was classified as likely pathogenic because it was found in a critical region, was not observed in healthy individuals, in silico splice effect prediction algorithms supported pathogenicity, and the patient's clinic was highly specific for LKENP. The compound pathogenic variant and the overlapping phenotype conducted us to reporting the novel variant as the causative compound heterozygous variant. The patient's son and her mother, her only living parent, were identified as carriers by Sanger sequencing analysis (Figure 2).

DISCUSSION

In the literature, it has been reported that causative variants of the *AARS2* gene lead not only to adult-onset leukoencephalopathy but also to clinical findings such as infantile mitochondrial cardiomyopathy, fatal primary pulmonary hypoplasia, fatal non-immune hydrops-fetalis, ovarian-leukodystrophy, retinopathy/optic atrophy. The clinical presentation of our patient was characterized by cognitive decline, spasticity

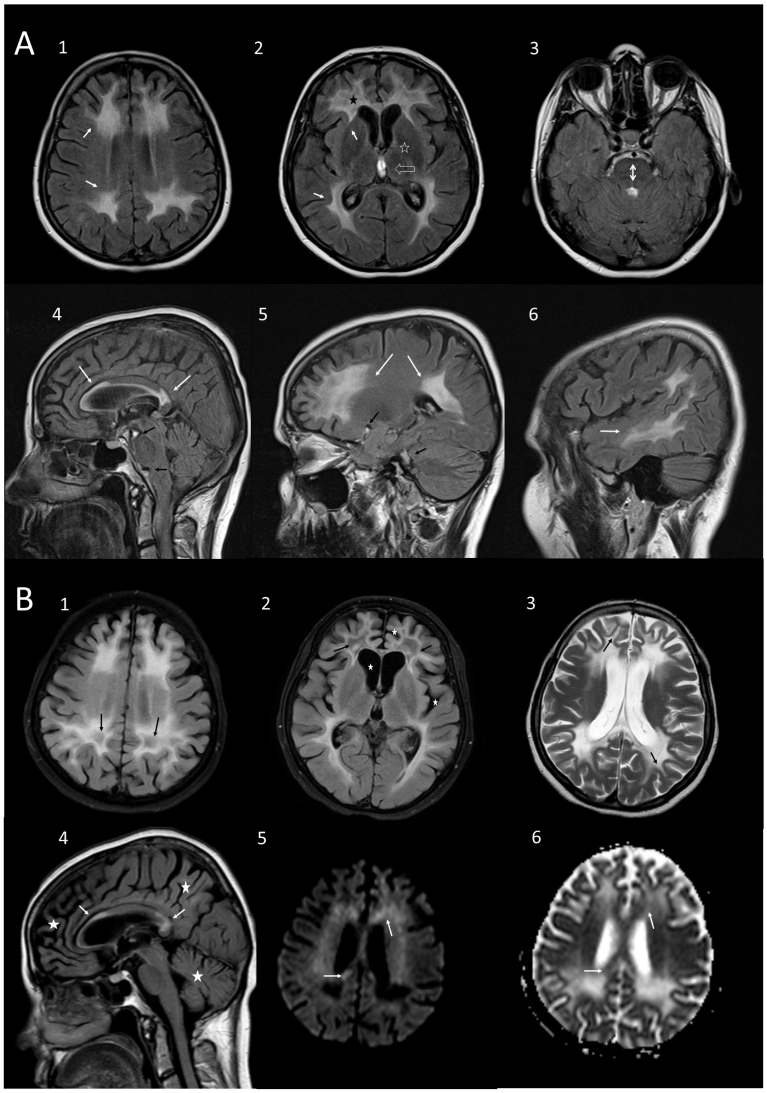

Figure 1. Cranial MRI of the patient performed 9 months prior to admission to our clinic (A), axial (1-3) and sagittal (4-6) FLAIR images. 1: At the centrum semiovale level, image shows confluent abnormally increased signal in periventricular and deep white matter including the subcortical U-fibers in bilateral frontal and parietal lobes (white arrows). 2: At the lateral ventricular level, image shows mildly low signal inside the increased signal at the forceps minor that corresponds to the rarefaction of the white matter (black asterix). Basal ganglions are spared (white asterix). Hemorrhagic high signal in the 3rd ventricle (open arrow). 3: Hemorrhagic high signal in the basal cisterns (double arrow). 4: Abnormal high signal in corpus callosum affecting the genu and splenium portions and reaching to the outer wall. Also there is high signal at the callososeptal interface (white arrows). Hemorrhagic high signal in the basal cisterns (black arrows). 5, 6: Altered signal is seen in the frontal, parietal and temporal subcortical white matter, notice that the corona radiata and basal ganglia are spared. Hemorrhagic high signal in the basal cisterns (black arrows). Follow up MRI of the patient repeated at our center (B), axial FLAIR (1, 2), axial T2W (3), sagittal FLAIR (4), DWI and ADC map (5, 6). 1: In bilateral parietal subcortical white matter, remarkable signal loss occurred that represents white matter rarefaction. 2: Signal loss in forceps minor (black arrow) and more obvious atrophy in frontal lobe and enlarged subarachnoid spaces and lateral ventricules (asterix). 3: Involvement of the subcortical U-fibers is demonstrated (black arrows). 4: Significant atrophy and reduced abnormal high signal in corpus callosum (white arrows). Significant atrophy is also shown in frontal-parietal lobes and cerebellar vermis (asterix). 5, 6: Patchy, linear and dot-like bilateral abnormal high signal on DWI images, but no accompanying low signal on ADC values (white arrow)

Table 1: Criteria used in evaluating the novel variant

Criteria	Explanation
PM1	Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation
PM2	Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)
PP4	Patient's phenotype or family history is highly specific for a disease with a single genetic etiology

and gait impairment which was also reported in the previous cases of *AARS2* leukoencephalopathy.[4]

In our patient, the frontoparietal predominant, confluent hyperintense T2W signal abnormalities in deep and periventricular white matter were detected.[4] One of the most striking findings was the rarefied areas in abnormal white matter that show the low signal in FLAIR images. Unlike fluid-filled, cystic white matter changes in the vanishing white matter disease, the rarefaction represents incomplete vanishing of the white matter. In vanishing white matter disease the low signal in the FLAIR and T1W images is expected to be as low as the CSF signal due to the cystic changes, however, mildly affected cases may show rarefaction until the late stages of the disease. *AARS2-L* imaging findings may also resemble adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP).[5] However, in our patient, there was subcortical U-fiber involvement which is unexpected in patients with ALSP. A publication including 10 *AARS2* subjects, involvement of U-fibers was not detected. Some features in diffusion-weighted imaging (DWI) may also present substantial clues regarding the diagnostic process. Such that, diffusion restriction or persistent diffusion alterations may suggest highly an underlying

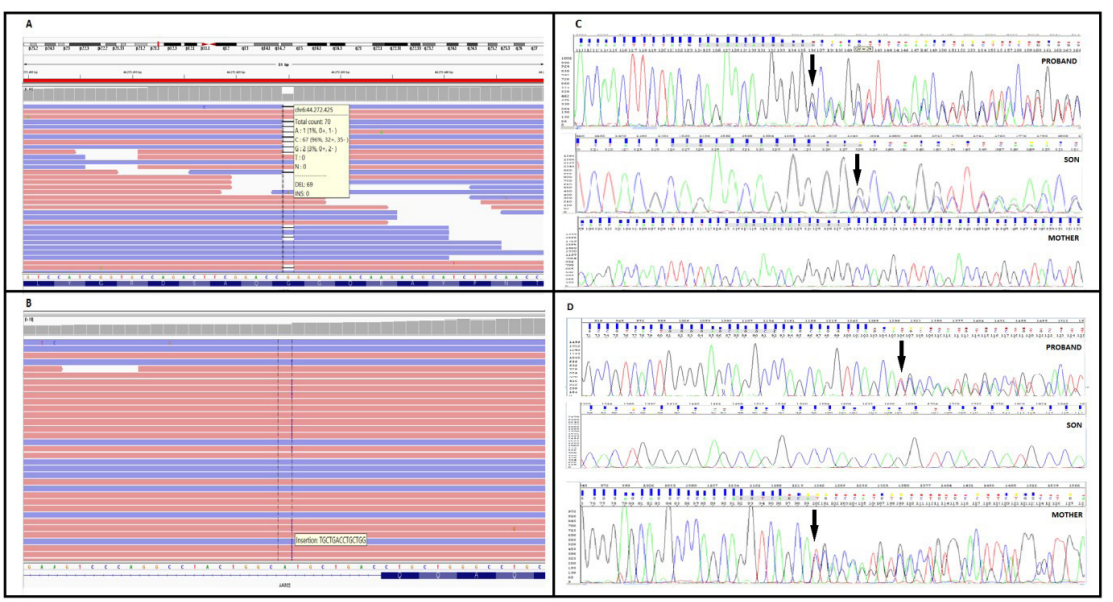

Figure 2. By NGS reads aligned with the hg19 reference sequence; the c.1709delG pathogenic variant (A) and the c.2358_2364 + 7dupCCAGCAGGTCAGCA likely pathogenic variant (B) were identified as compound heterozygous in the *AARS2* gene. (C) Sanger sequencing chromatogram showed that the proband and her son carried in the *AARS2* gene the c.1709delG variant as heterozygously, while the proband' s mother didn't carry. (D) Sanger sequencing chromatogram showed that the proband and her mother carried the c.2358_2364+7dupCCAGCAGGTCAGCA variant heterozygously in the *AARS2* gene while the proband's son didn't carry the mentioned mutation. The arrows indicate the heterozygous mutation sites.

ALSP or leukoencephalopathy with brain stem and spinal cord involvement and high lactate as the cause of leukoencephalopathy.[4,5] However, the DWI in our patient did not show a "true restriction" to aid in the diagnostic process.

The clinical and radiological findings observed in our patient are not specific to LKENP and may overlap with other types of leukoencephalopathy and many diseases caused by different etiologies.

In conclusion, we report here a case of LKENP with compound heterozygous variants. In the patient's history; She had a history of secondary amenorrhea at the age of 17, forgetfulness and depression in her 40s, progressive decline in motor/mental abilities and dementia. It was remarkable that the patient, whose MRI was reported as leukoencephalopathy, could not be diagnosed for years. Definitive diagnosis of the disease was possible with the use of the NGS-based targeted multigene panel. One of the causative variants detected in the genetic analysis examined was previously reported as pathogenic. The other causative variant had no functional evidence of pathogenicity reported based on the published literature and clinical database and was novel. Because, the related phenotype is reported as autosomal recessive and the compound variant is definitely pathogenic, it is hypothesized that overlapping phenotype and pathogenic prediction of in silico splice algorithms support us due to the molecular etiopathogenesis. We believe that, further investigation is needed for the functional effect of the variation; but lack of functional evidence is not a limitation of diagnosis. Reporting such variants could be useful for both routine diagnosis and research studies.

DISCLOSURE

Conflicts of interest: None

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