Meta Gene 30 (2021) 100953

Contents lists available at ScienceDirect

Meta Gene

journal homepage: www.elsevier.com/locate/mgene

Whole exome sequencing identified a novel homozygous *ARV1* mutation in an Iranian family with developmental and epileptic encephalopathy-38

Emran Esmaeilzadeh [a], Sahar Bayat [b], Reza Mirfakhraie [c], Milad Gholami [d,*]

[a] Fetal Health Research Center, Hope Generation Foundation, Tehran, Iran
[b] Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[c] Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[d] Department of Biochemistry and Genetics, School of Medicine, Arak University of Medical Sciences, Arak, Iran

ARTICLE INFO

Keywords:
Whole exome sequencing
ARV1 gene
Mutation
Developmental and epileptic encephalopathy-38

ABSTRACT

Developmental and epileptic encephalopathy-38 (DEE38) is an inherited neurodegenerative disorder described by the onset of various type of seizures usually between around 4 and 7 months of age. Mutations in the *ARV1* gene have recently been described in association with DEE38. Extracted genomic DNA from blood sample was used to perform whole exome sequencing in an affected member of an Iranian family with Developmental and Epileptic Encephalopathy type 38. The mutational screening revealed a novel homozygote *ARV1* gene mutation c.593_594delTT (p.Ile198MetfsTer4) in the proband. We identified a novel homozygous deletion in the *ARV1* that associates with the Developmental and epileptic encephalopathy-38.

1. Introduction

The term "Developmental and Epileptic Encephalopathy" (DEE) includes a group of disorders, all of which are characterized by early-onset seizures, electroencephalographic anomalies and developmental delay (Hamdan et al., 2017). DEE type 38 is a rare disorder inherited as an autosomal recessive trait (EIEE38, MIM 617020). Developmental and epileptic encephalopathy-38 (DEE38) is characterized by epileptic encephalopathy, intractable seizures, status epilepticus, severe developmental delay, profound intellectual, ataxia, dystonia and peripheral hypertonia (Palmer et al., 2016). Its prevalence is unknown, although the incidence of DEE has not been directly measured for distinct types. Still, the incidence is 3–5% and 6.1–8.2% of all epilepsies developed in the first year and within the three years of life, respectively (Kural and Ozer, 2012). This severe condition may lead to sudden death due to aspiration or stubborn epilepsy in some patients with early-onset symptoms (Nilsson et al., 1999).

Among the numerous genes involved in epilepsy, ACAT related enzyme 2 required for viability 1, *ARV1*,(MIM 611647) is the most clinically related gene with reported mutations detected in patients suffering from DEE38 (Alazami et al., 2015). *ARV1*, also known as DEE38 and EIEE38, is located at chromosome 1q42.2 and encodes two non-coding RNAs (ncRNAs) and three coding-mRNAs. The coding-mRNAs are weakly expressed in human tissues and encode a lipid transporter in the endoplasmic reticulum (ER), which is involved modulating the fatty acid homeostasis process. The *ARV1* gene is composed of 6 exons which five exons encode for a 271 amino acids protein that plays an important role in the normal sphingolipid metabolism (Lagor et al., 2015). ARV1 is characterized by an N-terminal conserved motif composed of a cytosolic zinc ribbon motif or 'Arv1 homology domain', (AHD) followed by some transmembrane domains and a large loop inside the ER lumen with the C-terminus of the protein expanding back into the ER lumen (Villasmil and Nickels Jr, 2011).The association of ARV1 deficiency with epileptic encephalopathy is well documented. It seems that the ARV1 function in human is unknown, however, it is involved in flipping the GPI (Glycosyl-phosphatidylinositol) precursors into the ER lumen, bile acid metabolism, intracellular sterol transportation, metabolism and transportation of cholesterol, regulation of plasma membrane sterol distribution and sphingolipid and sterol metabolism in yeast (Swain et al., 2002). The correction of gene deficiency by human ARV1 in yeast led to the suggestion that the human and yeast *ARV1* genes may have similar roles during development (Palmer et al., 2016).

The critical role of ARV1 protein in the pathogenesis of DEE38 has been supported in previous studies (Alazami et al., 2015; Davids et al., 2020; Segel et al., 2020). Several findings have shown that mutations in

* Corresponding author.
E-mail address: dr.gholami@arakmu.ac.ir (M. Gholami).

https://doi.org/10.1016/j.mgene.2021.100953
Received 19 June 2021; Accepted 18 July 2021
Available online 21 July 2021
2214-5400/© 2021 Elsevier B.V. All rights reserved.

the *ARV1* can disrupt sterol transportation from the ER and causes reduced expression levels of GPI-anchored proteins (Davids et al., 2020; Segel et al., 2020). In the current study, we describe the results of whole exome sequencing in an Iranian family with DEE38.

2. Material and methods

The family with developmental and epileptic encephalopathy-38 comprised of two affected members from a consanguineous marriage (Fig. 1.A). Informed consent was obtained from the parents. The Medical Ethics Committee of the Arak University of Medical Sciences permitted this study (Approval no: IR.ARAKMU.REC.1399.264).

Genomic DNA (gDNA) from the proband and her parents was isolated from peripheral blood samples using the salting out method. One μg of gDNA from proband was sheared, and exome capture was carried out using SureSelectXT2 V6 Exome. The libraries were sequenced to mean > 80-100× coverage on Illumina sequencing platform. The sequences obtained were aligned to the human reference genome (GRCh37/hg19) using BWA program and analyzed using Picard and GATK version 3.6 to identify variants relevant to the clinical indication. We follow the GATK best practices framework for the identification of variants in the sample. Gene annotation of the variants is performed using VEP program against the Ensemble release 87 human gene model. Clinically relevant mutations were annotated using published variants in the literature and diseases databases – ClinVar, OMIM, GWAS, HGMD and SwissVar. Common variants are filtered based on the allele frequency in 1000 Genome Phase 3, ExAC, EVS, dbSNP147, 1000 Japanese Genome and Iranome. Non-synonymous variants effects were calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2, Mutation Assessor and LRT. Finally, for variant validation and segregation analysis, polymerase chain reaction (PCR) was performed to amplify the targeted variants using specific primers (F 5′AGGGGA TATATTTTGGATGGGGA3′ and R-5′AACAAACACTGAGTACCTGCT3′). The PCR products were then subjected to Sanger sequencing on an automated ABI PRISM 3130XL (Applied Biosystems, USA).

3. Results

The proband (IV-2), was born to healthy Iranian consanguineous parents originating from Kurdish ethnicity and presented with clinical indications of seizures, microcephaly and abnormal MRI findings. Her elder sibling died at the age of 10 months with similar symptoms.

A novel homozygous two base pair deletion in exon 4 of the *ARV1* gene (chr1:231131650_231131651delTT; c.593_594delTT, Depth: 107×) was detected that results in a frameshift mutation and protein premature truncation (p.Ile198MetfsTer4; ENST00000310256). The observed variant has not been reported in the 1000 genomes, ExAC and our Iranome databases. Due to the *in silico* prediction tools and InterVar classifying system, the variant was found to be damaging. The parents were heterozygote carriers for the deletion and did not present any symptoms of the disease. Based on the above evidence, this *ARV1* variation is classified as a variant of likely pathogenic.

4. Discussion

Whole exome sequencing (WES) is now extremely strong and affordable. It is becoming more labor-and cost-efficient to prefer WES to other means of screening, particularly in rare diseases with a recessive pattern. Using WES of the known *ARV1* gene in a DEE38 patient, we found a new nonsense variant (p.Ile198MetfsTer4) and we give a definite molecular diagnosis for her. Her elder sibling (Fig. 1.A) exhibited similar clinical features, including seizures, microcephaly and abnormal MRI findings at the age of 10 months.

The molecular bases of the DEE38 are related to defective sterol

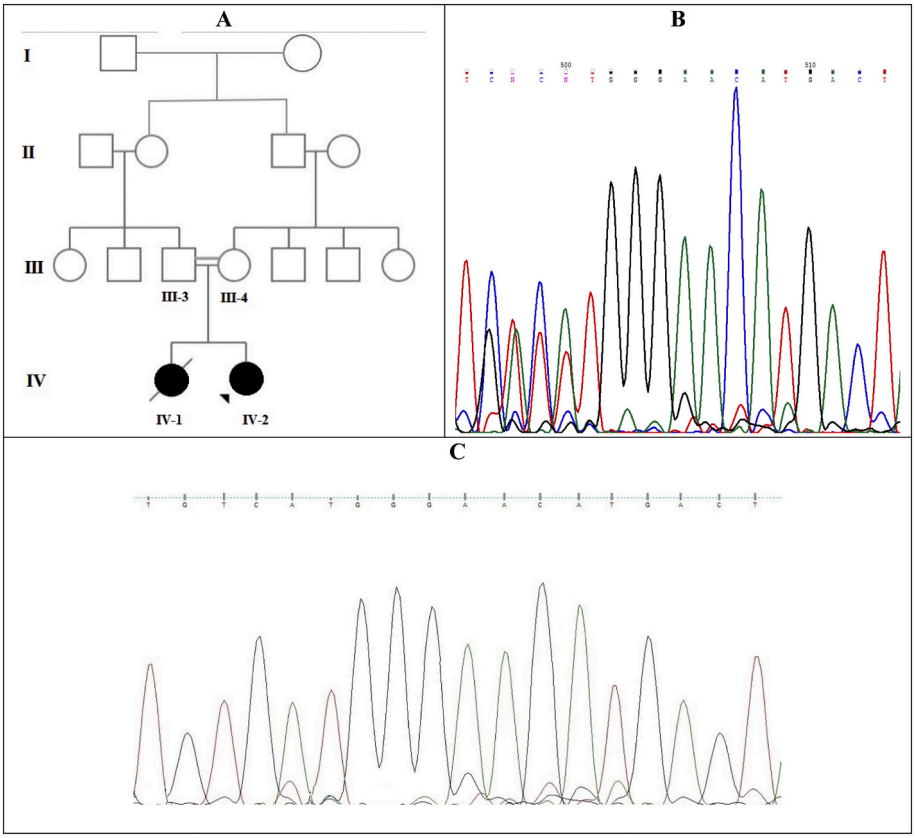

Fig. 1. A) The family pedigree of Developmental and Epileptic Encephalopathy type 38. B & C) The mutation status of *ARV1* (c.593-594delTT) was recognized by the Sanger sequencing, the patient (IV-2) was homozygous for c.593-594delTT and her parents (III-3 & III-4) were in the heterozygous state.

movement from ER (Georgiev et al., 2013). Mutations of the *ARV1* gene have been reported in DEE38 (Henneberry and Sturley, 2005). Also, deletions within the Arv1 gene results in weight loss, fat mass and plasma lipids in mice, even subjects consumed a greater total of food (Lagor et al., 2015). Moreover, ARV1 deficiency leads to weight loss and feeding problems in human that is curable using GI-tube feeding (Palmer et al., 2016).

Lack of Arv1 in mice CNS cells resulted in an augmented locomotor response or changed neuronal signaling to the muscle and seizure, similar to what is described in patients with ARV1 deletion (Lagor et al., 2015).

Alazami et al. have reported a homozygous pathogenic variant in human *ARV1* gene in 3 subjects with epileptic encephalopathy. They identified the p.Gly189Arg variant in 2 siblings and their cousin with intellectual disability, visual loss and "neurodegenerative disease" (Alazami et al., 2015). Contrary to the first report of DEE38 in a patient with neurodegeneration symptoms and blindness (Alazami et al., 2015), none of the patients in another report showed neurodegeneration or visual impairment (Nashabat et al., 2019). Interestingly, Nashabat et al. observed that all individuals with ARV1 mutation developed ataxia, which was not described before (Nashabat et al., 2019). In another report by Palmer et al., five patients from one family showed severe neurodevelopmental delay, movement disorder, visual dysfunction and infantile-onset seizure disorder. In the mentioned study, a homozygous splice site mutation (Lys59_Asn98del variant) in the *ARV1* was detected, and all subjects died by the age of 5 years. Furthermore, they detected a homozygous *ARV1* c.674-2A > T splice variant in two siblings from another family. Palmer et al. observed that Arv1 knockout mice shows the lean phenotype, decreased body mass, unusual circling behavior, seizures, and decreased survival compared to controls (Palmer et al., 2016).

Seven additional patients with profound intellectual disability, severe developmental delay, central hypotonia, peripheral hypertonia, feeding problems, seizures, and cortical blindness from two distinct families were recently described by Davids et al. They identified two biallelic splice site variants c.674-2A>T (p.Thr266-Phe271del) and c.294+1 G > A (p.Lys59-Asn98del) in *ARV1* gene, in family 1 and family 2, resulting in exon skipping and severe symptoms. Abnormal splicing of ARV1gene and its reduced expression leads to epilepsy of infancy, developmental delays, hypotonia, low visual function and cerebellar atrophy in patients (Davids et al., 2020).

A homozygous missense variant in ARV1 (c.565 G > A, p.Gly189Arg) first reported by Alazami in a patient of consanguineous pedigree and is characterized by the intellectual disability, seizures and ataxia. According to another study, a family with two affected brothers who carry the ARV1 p.Gly189Arg variant has been associated with a milder DEE38 phenotype (Segel et al., 2020). Interestingly, even within families with the same genetic alteration, the affected patients show different clinical characteristics. This may be associated with the region of mutation and the importance of the impaired domains. It is reported that mutations localized at the ARV1 homology domain (AHD) domain were related to severe form of DEE38 than the mutations localizing at the transmembrane domains (Davids et al., 2020). For example, the loss of exon 5 leads to the deletion of a forecasted helical transmembrane domain that affects correct ARV1 localization to the ER. Also, the loss of exon 2, which encodes the AHD, may lead to a more severe disease due to disturbing the ARV1 function in lipid homeostasis maintenance (Tinkelenberg et al., 2000; Forés et al., 2006).

In conclusions, we report a female individual with a homozygous frameshift deletion in the *ARV1* gene. This frameshift deletion truncates the protein transmembrane domains and removes over half of the Arv1 protein.

Funding

This work was supported by the Research Council of Arak University of Medical Sciences (Grant Number: 3802) for financial support.

Ethics approval

The Medical Ethics Committee of the Arak University of Medical Sciences approved this study (approval no: IR.ARAKMU.REC.1399.264).

Author contribution

EE, MG, SB and RM: Designed the research study, performed the experiments, sample, and data collection. EE, MG and RM: Analyzed data, writing the manuscript and assisted in drafting the manuscript. All authors approved the final manuscript.

Declaration of Competing Interest

Authors do not have any conflict of interest to disclose.

Acknowledgment

We would like to thank all the patients and families, for their participation in this research.

References

Alazami, A.M., Patel, N., Shamseldin, H.E., et al., 2015. Accelerating novel candidate gene discovery in neurogenetic disorders via whole-exome sequencing of prescreened multiplex consanguineous families. Cell Rep. 10 (2), 148–161.

Davids, M., Menezes, M., Guo, Y., et al., 2020. Homozygous splice-variants in human ARV1 cause GPI-anchor synthesis deficiency. Mol. Genet. Metab. 130 (1), 49–57.

Forés, O., Arró, M., Pahissa, A., et al., 2006. Arabidopsis thaliana expresses two functional isoforms of Arvp, a protein involved in the regulation of cellular lipid homeostasis. Biochim. Biophys. Acta 1761 (7), 725–735.

Georgiev, A.G., Johansen, J., Ramanathan, V.D., et al., 2013. Arv1 regulates PM and ER membrane structure and homeostasis but is dispensable for intracellular sterol transport. Traffic 14 (8), 912–921.

Hamdan, F.F., Myers, C.T., Cossette, P., et al., 2017. High rate of recurrent de novo mutations in developmental and epileptic encephalopathies. Am. J. Hum. Genet. 101 (5), 664–685.

Sterol homeostasis in the budding yeast, *Saccharomyces cerevisiae*. In: Henneberry, A.L., Sturley, S.L. (Eds.), 2005. Semin Cell Dev Biol. Elsevier.

Kural, Z., Ozer, A.F., 2012. Epileptic encephalopathies in adults and childhood. Epilepsy Res. Treat. 2012.

Lagor, W., Tong, F., Jarrett, K., et al., 2015. Deletion of murine Arv1 results in a lean phenotype with increased energy expenditure. Nutr. Diabet. 5 (10) (e181-e).

Nashabat, M., Al Qahtani, X.S., Almakdob, S., et al., 2019. The landscape of early infantile epileptic encephalopathy in a consanguineous population. Seizure 69, 154–172.

Nilsson, L., Farahmand, B., Persson, P., et al., 1999. Risk factors for sudden unexpected death in epilepsy: a case control study. Lancet 353 (9156), 888–893.

Palmer, E.E., Jarrett, K.E., Sachdev, R.K., et al., 2016. Neuronal deficiency of ARV1 causes an autosomal recessive epileptic encephalopathy. Hum. Mol. Genet. 25 (14), 3042–3054.

Segel, R., Aran, A., Gulsuner, S., et al., 2020. A defect in GPI synthesis as a suggested mechanism for the role of ARV1 in intellectual disability and seizures. Neurogenetics 21 (4), 259–267.

Swain, E., Stukey, J., McDonough, V., et al., 2002. Yeast cells lacking the ARV1 gene harbor defects in sphingolipid metabolism complementation by human ARV1. J. Biol. Chem. 277 (39), 36152–36160.

Tinkelenberg, A.H., Liu, Y., Alcantara, F., et al., 2000. Mutations in yeast ARV1 alter intracellular sterol distribution and are complemented by human ARV1. J. Biol. Chem. 275 (52), 40667–40670.

Villasmil, M.L., Nickels Jr., J.T., 2011. Determination of the membrane topology of Arv1 and the requirement of the ER luminal region for Arv1 function in Saccharomyces cerevisiae. FEMS Yeast Res. 11 (6), 524–527.