RARS2 mutations cause early onset epileptic encephalopathy without ponto-cerebellar hypoplasia

Nishri Daniella ^{1,2*}, Goldberg-Stern Hadassa ^{3,8*}, Noyman Iris ⁴, Blumkin Lubov ^{1,8}, Kivity Sara ^{1,3}, Saitsu Hirotomo⁵, Nakashima Mitsuko⁵, Matsumoto Naomichi⁵, Leshinsky-Silver Esther^{1,7,8}, Lerman- Sagie Tally ^{1,8}, Lev Dorit ^{1,6,8}

¹ Metabolic-Neurogenetic Clinic, Wolfson Medical Center, Holon, Israel

² Child Development Center, Central District, Maccabi Health Services, Tel Aviv, Israel

³ Epilepsy center, Schneider's Children Medical Center, Petah Tiqwa, Israel

⁴ Pediatric Neurology Unit, Soroka Medical Center, Beer-Sheba, Israel

⁵. Department of Human Genetics, Graduate School of Medicine, Yokohama City University, Yokohama, Japan

⁶Institute of Medical Genetics, Wolfson Medical Center, Holon, Israel

⁷ Molecular Genetics Laboratory, Wolfson Medical Center, Holon, Israel

⁸Sackler School of Medicine, Tel-Aviv University, Tel-Aviv,

*both authors contributed equally to the manuscript

Corresponding author: Dr Dorit Lev

Institute of Medical Genetics

Wolfson Medical Center

POB 5, Holon 58100

Israel

Telephone: 972-3-5028536

Fax: 972-3-5028566

E-mail: dorlev@post.tau.ac.il

ABSTRACT

Introduction : Early onset epileptic encephalopathies (EOEEs) are a group of devastating diseases, manifesting in the first year of life with frequent seizures and/or prominent interictal epileptiform discharges on the electroencephalogram, developmental delay or regression and usually a poor prognosis. There are numerous causes for EOEEs making the diagnostic workup time consuming and costly.

Methods: We describe two siblings with fatal EOEE, profound global developmental delay and post- natal microcephaly that underwent extensive biochemical and metabolic workup in vain. Neuro-imaging disclosed non-specific progressive cerebral atrophy.

Results: Whole-exome sequencing (WES) disclosed compound heterozygous mutations in the gene encoding for mitochondrial arginyl-transfer RNA synthetase, *RARS2*. This gene has been previously described as the cause of pontocerebellar hypoplasia type 6.

Conclusion: We suggest that *RARS2* gene mutations can cause a metabolic neurodegenerative disease manifesting primarily as EOEE with post-natal microcephaly, without the distinctive radiological features of pontocerebellar hypoplasia.

Key words: epileptic encephalopathy, *RARS2*, mitochondrial, pontocerebellar hypoplasia

1. Introduction

Early onset epileptic encephalopathies are a group of devastating diseases manifesting in the first year of life with frequent seizures and/or prominent interictal epileptiform discharges on the electroencephalogram (EEG), developmental delay/regression and usually a poor prognosis.¹ There are many etiological causes for EOEEs: structural brain abnormalities, metabolic, related to recognizable dysmorphic syndromes, chromosomal, monogenic, or environmental.¹

Next-generation sequencing, especially whole-exome sequencing (WES), is becoming a cost-effective mean to elucidate the molecular basis of EOEEs. Recently, Veeramah and colleagues found de-novo mutations in genes of known or plausible clinical significance to neuronal excitability in seven out of ten children with severe epilepsy.²

Saitsu and colleagues are conducting a comprehensive genetic analysis for EOEEs (work in progress).

Here we describe two siblings with fatal EOEE, profound global developmental delay (GDD) and post-natal microcephaly due to mutations in the gene encoding for mitochondrial arginyl-transfer RNA (tRNA) synthetase *RARS2*, found through this collaboration. Extensive biochemical and metabolic workup in both children was normal. We did not suspect *RARS2* mutation beforehand since neuro-imaging did not disclose pontocerebellar hypoplasia (PCH), and lactate levels were normal in blood, urine and magnetic resonance spectroscopy (MRS).

2. Cases history

The siblings were born to unrelated parents: Moroccan Jewish mother and Bukhara–Libyan Jewish father. There is no family history of neurological diseases. (Figure 1 panel A) Both were born at term after a normal pregnancy and delivery. Head circumference at birth was normal.

Patient II-3 was noted to have poor eye contact from birth. At the age of two months, after receiving the HIB-OPV-DTaP vaccination, she became apathetic and irritable. One week later, she developed multiple brief seizures manifesting as eye blinking and clonic movements. Phenobarbital was started and the seizures abated for two months. When resumed, she had focal, multifocal and myoclonic seizures which did not respond to multiple antiepileptic drugs (AEDs) and the ketogenic diet. She lost all developmental milestones. An initial EEG was reported as normal, but later EEG showed a left anterior temporal focus. At the age of four months, EEG showed bursts of spikes and poly-spikes intermixed with high slow waves and decrements.

Brain magnetic resonance imaging (MRI) at two and a half months was reported as normal. MRI at 16 months showed diffuse brain atrophy, more prominent in the frontal regions, with cerebellar atrophy. The pons and brainstem were intact [Figure 1 panel B]. MRS of white matter disclosed normal signals.

An extensive biochemical and metabolic work-up was normal.

Cerebrospinal fluid (CSF) analysis for biogenic amines showed low levels of 5-methylenetetrahydrofolate and high titers of anti-folate receptor antibodies. Folinic acid therapy was started, with no improvement.

Muscle biopsy, including mitochondrial respiratory chain enzymes activity, and liver biopsy showed no abnormalities.

Prior to her demise at the age of three years, she was microcephalic, in a vegetative state with hyperactive reflexes. Seizures remained intractable, mainly myoclonic and multifocal, aggravated by fever, with multifocal, migratory discharges on EEG.

Patient II-4 developed seizures at the age of three months, during a febrile illness, manifesting as eye blinking and clonic movements. Head circumference was 40.3 cm (50th percentile). He

demonstrated visual inattention and had no social smile. He had axial hypotonia with limb hypertonia and brisk deep tendon reflexes.

Initial EEG showed a left fronto-temporal focus. Phenobarbital was started and he was seizures-free for six months. At that point, he developed intractable epilepsy (myoclonic seizures aggravated by febrile illnesses and tonic seizures). He lost all developmental milestones. EEG showed bilateral epileptiform discharges, multifocal spikes and polyspikes. He underwent an extensive biochemical and metabolic workup, including CSF analysis, which was all within the normal range.

Head ultrasound at the age of three months was reported as normal. Brain MRI at age 13 months showed diffuse gray matter atrophy. The pons and cerebellum were intact. There was no lactate peak on MRS.

Neurological examination at the age of three and a half years showed spasticity of limbs. He passed away at four years of age.

3. Methods

Genomic DNA was captured using the SureSelect Human All Exon v5 Kit (50 Mb; Agilent Technologies, Santa Clara, CA) and sequenced on an Illumina HiSeq2000 (Illumina, San Diego, CA) with 101 bp paired-end reads. Exome data processing, variant calling, and variant annotation were performed as previously described.³ The *RARS2* mutations were validated by Sanger sequencing.

The parents signed an informed consent before DNA extraction.

4. Molecular analysis

WES was performed for the two affected siblings. The average mean read depth of the protein-coding regions of RefSeq genes for the siblings is 81.5 and 83.0 $\times$, such that 95.7 and 95.8% of target coding sequences were covered by 10 reads or more. We filtered out common single-nucleotide polymorphisms that met the following two criteria: variants showing minor

allele frequencies $\geq 1\%$ in database dbSNP 135 and variants found in more than three of our in-house 575 control exomes. We surveyed for genes possessing two heterozygous variants or a homozygous mutation in the two siblings, which were consistent with autosomal recessive model. Among 24 genes that met the above criteria, we found two possible splice site variants in *RARS2*: c.878+5G>T and c.110+5A>G in transcript variant 1 (NM_020320.3). These two mutations were not found in the 6,500 exomes sequenced by the National Heart, Lung, and Blood Institute exome project (ESP6500) or among our 575 in-house control exomes. Parental analysis revealed that the c.878+5G>T and the c.110+5A>G mutations were transmitted from their father and mother, respectively.

5. Discussion

We present two siblings with a neurodegenerative disease manifesting primarily as fatal EOEE with profound GDD, post-natal microcephaly and progressive cerebral atrophy due to mutations in *RARS2*.

The name Pontocerebellar Hypoplasia (PCH) originates from a report of Brun, in which he described human brain development and abnormalities associated with brain development. Later, Brouwer suggested that pontocerebellar hypoplasia is possibly due to degeneration rather than to hypoplasia and subsequent reports described the pathology as atrophy of cerebellar hemispheres with relative sparing of the flocculi and vermis and apparent fragmentation of the cerebellar dentate nucleus.⁹ Pontocerebellar Hypoplasia (PCH) is a group of very rare, inherited progressive neurodegenerative disorders with prenatal onset. Up to now seven different subtypes have been reported (PCH1-7).⁹

Since the first description of *RARS2* mutations as the molecular etiology for the phenotype of pontocerebellar hypoplasia (PCH) type 6 by Edvardson et al⁴, 15 other patients have been described (Table 1).⁵⁻¹⁰

Reviewing these 18 cases, a common phenotype emerges of early onset encephalopathy. All the patients presented in the immediate post-natal or early infantile period (age at onset: one day-four months). Epilepsy was present in 89% (16/18), and was consistent with EOEE in most cases (14/16, 87.5%). Many children had more than one seizure type, the most frequent were myoclonic (8/16, 50%) followed by clonic (5/16, 31.5%). The seizures were described as multifocal or alternating sides in 6/16 (37.5%). Initial EEG demonstrated slowing of the background in 10/16 (62.5%) and epileptiform discharges in 8/16. EEG was reported as initially normal in 3/16 (19%), but almost all the following recordings showed deterioration. Few patients demonstrated initial response to an AED (either phenobarbital or valproic acid) but eventually all but one had intractable epilepsy. In this single patient (sibling one, Kastrissianakis et al)⁵ the epilepsy was controlled by topiramate.

Regardless of their epilepsy status, all patients displayed profound GDD - none attained any significant milestones. Our patients had a short initial period of apparently normal development but later lost all motor milestones. Most patients (8/13, 61.5%) had normal occipito-frontal circumference (OFC) at birth, but almost all (13/14, 93%) developed postnatal microcephaly, up to -6 standard deviations. Neurological examination demonstrated axial hypotonia and limbs increased tone with brisk reflexes (or "spastic quadriparesis") in 11/16 (69%). One patient displayed dystonia. Almost all patients demonstrated visual inattention or central visual impairment and nearly all (10/12, 83%) had feeding difficulties resulting in gastrostomy insertion. The condition proved to be fatal in 8/16 (50%, range: six days-4 years).

Most patients (excluding our own) had elevated lactate levels either in blood, urine or MRS. Regarding neuro- imaging, the most common finding was progressive cerebral (15/15) and cerebellar (14/15, 93%) atrophy and NOT progressive pontocerebellar atrophy (PCA, present in 10/15, 67%). Of note is that normal pons and brainstem were evident on the last imaging

(range: 9 months-3 years) in our cases (Figure 1) and also in the two siblings described by Kastrissianakis et al.⁵ Scrutinizing the figures provided by Cassandrini et al⁶, we consider the pons of their patient B-01 to be of normal volume (hence progressive PCA apparent in 9/15, 60%).

Of note is that the c.110+5A>G mutation found in our patients is identical to the one described by Edvardson et al⁴, raising the possibility of a founder mutation in Moroccan Jews. From the cases described in the literature and our patients we cannot prove genotype-phenotype correlation, however all patients displayed a very severe disease with profound GDD and epilepsy and the major variability is in the neuroimaging phenotype ranging from severe pontocerebellar hypoplasia to normal pons.

Epilepsy is a common feature of mitochondrial diseases, described in up to 60% of individuals with biochemical confirmation. Seizures may be the presenting symptom of a mitochondrial disease, but in most cases the first seizures are preceded by other symptoms e.g. failure to thrive or developmental delay. Clinical recognition of mitochondrial epilepsy is difficult due to the broad clinical and biochemical picture. The genetic basis for mitochondrial epilepsy is highly heterogeneous and can roughly be divided into the following categories:

- (1) abnormal oxidative phosphorylation proteins e.g. *NDUFV1*, *NDUFS4*, *NDUFA1*;
- (2) disorders of assembly proteins e.g. *TMEM70* mutations;
- (3) disorders affecting mitochondrial import e.g. *SLC25A22* mutations;
- (4) disorders of mitochondrial DNA (mtDNA) maintenance e.g. *POLG*, *PEO1* mutations;
- (5) disorders in mitochondrial translation e.g. *RARS2*; and (6) coenzyme Q10 deficiency. The genetic defects can either be in the mtDNA or the nuclear genome.¹¹

RARS2 gene mutations were the first nuclear-encoded defects of mtDNA translation that were associated with early-onset intractable epilepsy and a neurodegenerative course.⁴

Recently, a similar phenotype was described due to mutations in two other mitochondrial aminoacyl-tRNA Synthetases (mt-aaRSs): *FARS2* encoding the mitochondrial phenylalanine tRNA synthetase and *QARS*, encoding glutaminyl-tRNA synthetase.¹²⁻¹⁴

FARS2 mutations have been identified by WES in two couples of siblings. The clinical picture included neonatal/infantile onset epileptic encephalopathy, liver disease, development arrest, post-natal microcephaly and lactic acidosis. Imaging of two patients disclosed severe progressive cortical atrophy with bilateral signal increase in the putamina and relative sparing of the cerebellum. One patient had a lactate peak on MRS. Neuropathological findings together with liver disease filled the criteria for Alpers-Huttenlocher syndrome (OMIM#614946). All the patients identified so far died within the first two years of life.¹²⁻¹³

QARS mutations have been identified by WES as the causative variants in two unrelated families affected by progressive microcephaly, severe neonatal/infantile onset epilepsy and profound GDD. Imaging disclosed atrophy of the cerebral cortex, cerebellar vermis and mild atrophy of the cerebellar hemispheres. Two siblings had congenital microcephaly (ranging (-)2.1 to (-) 3.5SD) that progressed significantly (up to (-)10.4SD); the other two had relatively mild post-natal microcephaly. The seizures were polymorphic and of alternating/migrating pattern.¹⁴ One patient displayed visual inattention. Loss of *qars* function in the zebrafish model causes extensive cell death at later stages of development.¹⁴

The aaRSs are key enzymes in the translation of genetic information. They catalyze the specific attachment of each of the 20 amino acids to a cognate tRNA. Mt-aaRSs are encoded by nuclear genes and imported into the mitochondria. In general, mutations in *aaRS2* genes have been associated with diverse clinical presentations, usually with an early onset and transmitted as autosomal recessive traits. The clinical variability and tissue specificity of the disorders associated with aaRSs mutations are remarkable and not yet explained.¹²⁻¹³

6. Conclusion

This report broadens the phenotypic spectrum of *RARS2* gene mutations and suggests that *RARS2* mutations can cause a metabolic neurodegenerative disease manifesting primarily as EOEE with post-natal microcephaly without the distinctive radiological features of PCH.

Acknowledgements

This work was supported by a Grant-in-Aid for the Ministry of Health, Labour and Welfare of Japan; Grants-in-Aid for Scientific Research (B) (25293085) and (A) (13313587), and challenging Exploratory Research (26670505) from the Japan Society for the Promotion of Science; the Takeda Science Foundation; the fund for Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program in the Project for Developing Innovation Systems from the Japan Science and Technology Agency; the Strategic Research Program for Brain Sciences (11105137); and a Grant-in-Aid for Scientific Research on Innovative Areas (Transcription Cycle) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (12024421).

Table 1: clinical and radiological spectrum of 18 patients with *RARS2* mutations

Figure 1: Patients pedigree and neuro imaging

References

1. Kamien BA, Cardamone M, Lawson JA et al. A genetic diagnostic approach to infantile epileptic encephalopathies. *J Clin Neurosci*. 2012;19(7):934-41.
2. Veeramah KR, Johnstone L, Karafet TM et al. Exome sequencing reveals new causal mutations in children with epileptic encephalopathies. *Epilepsia*. 2013;54(7):1270-81.

3. Saitsu H, Nishimura T, Muramatsu K et al. *De novo* mutations in the autophagy gene *WDR45* cause static encephalopathy of childhood with neurodegeneration in adulthood. *Nat Genet.* 2013;45:445-9.
4. Edvardson S, Shaag A, Kolesnikova O et al. Deleterious mutation in the mitochondrial arginyl-transfer RNA synthetase gene is associated with pontocerebellar hypoplasia. *Am J Hum Genet.* 2007;81:857-62.
5. Kastrissianakis K, Anand G, Quaghebeur G et al. Subdural effusions and lack of early pontocerebellar hypoplasia in siblings with RARS2 mutations. *Arch Dis Child.* 2013;98(12):1004-7.
6. Cassandrini D, Cilio MR, Bianchi M et al. Pontocerebellar hypoplasia type 6 caused by mutations in RARS2: definition of the clinical spectrum and molecular findings in five patients. *J Inherit Metab Dis.* 2013;36(1):43-53.
7. Glamuzina E, Brown R, Hogarth K et al. Further delineation of pontocerebellar hypoplasia type 6 due to mutations in the gene encoding mitochondrial arginyl-tRNA synthetase, RARS2. *J Inherit Metab Dis.* 2012;35(3):459-67.
8. Rankin J, Brown R, Dobyns WB et al. Pontocerebellar hypoplasia type 6: A British case with PEHO-like features. *Am J Med Genet A.* 2010;152A(8):2079-84
9. Namavar Y, Barth PG, Kasher PR et al. Clinical, neuroradiological and genetic findings in pontocerebellar hypoplasia. *Brain.* 2011;134(Pt 1):143-56.
10. Joseph JT, Innes AM, Smith AC et al. Neuropathologic features of pontocerebellar hypoplasia type 6. *J Neuropathol Exp Neurol.* 2014 Nov;73(11):1009-25.
11. Rahman S. Mitochondrial disease and epilepsy. *Dev Med Child Neurol.* 2012;54(5):397-406.
12. Diodato D, Ghezzi D, Tiranti V. The Mitochondrial Aminoacyl tRNA Synthetases: Genes and Syndromes. *Int J Cell Biol.* 2014;2014:787956. Epub.

13. Elo JM, Yadavalli SS, Euro L et al. Mitochondrial phenylalanyl-tRNA synthetase mutations underlie fatal infantile Alpers encephalopathy. *Hum Mol Genet.* 2012 15;21(20):4521-9.
14. Zhang X, Ling J, Barcia G et al. Mutations in QARS, encoding glutaminyl-tRNA synthetase, cause progressive microcephaly, cerebral-cerebellar atrophy, and intractable seizures. *Am J Hum Genet.* 2014 3;94(4):547-58.

Table 1: clinical and radiological spectrum of 18 patients with *RARS2* mutations

Patient	Total (N=18)
Clinical Feature	
Sex	F 10/17 M 7/17
Perinatal and neonatal	Uneventful 4/16 SGA 3/16 Hypoglycemia 4/16 Apnea 3/16 Hypotonia 2/16
Mutation	coH 14/17
Initial symptoms	Encephalopathy 4/15 Visual inattention 2/15 Abnormal movements 2/15 Poor feeding 4/15 Hypotonia 1/15 Apnea 2/15
<u>Epilepsy</u>	
Age at onset	1 st d-4 mo
Seizure types & semiology	recurrent apnea 5/16 myoclonic 8/16 clonic 5/16 tonic 2/16 tonic clonic 1/16 multifocal/alternating sides 6/16 SE 2/16 No specification 5/16
Epileptic syndrome	EOEE 14/16 intractable epilepsy 10/11
Initial EEG	N 3/16 Slowing of background 10/16 Epileptiform discharges 8/16
Evolution of EEG	6/7
<u>Development and neurological</u>	
OFC at Birth	N 8/13 Borderline Mic 3/13 Mic 2/13
OFC at Last follow up	Progressive Mic 13/14
Neurological exam	Initial N 2/16 Initial hypotonia 10/16 Axial hypotonia and limb increased tone/spastic QP 11/16 Contractures 5/16 Dystonia 1/16
Development/Intellectual disability degree	no milestones 13/13
Ophthalmologic /visual Features including VEP and ERG	visual inattention/CVI 12/14 N Fundus 6/7 optic atrophy 1/7 N VEP+ERG 3/4 Abnormal VEP 1/4
Feeding difficulties resulting in gastrostomy	10/12

Status at last follow up	Mortality 8/16 intractable epilepsy 7/16
Investigations	
Elevated lactate (blood, urine, CSF or peak in MRS)	Elevated lactate (anywhere): 12/15
Muscle biopsy	Reduced Enzyme activities of MRC (any complex, muscle/fibroblasts) 6/11
Neuro imaging	Neuro imaging 17/18 Initially N (including fetal) 8/17 Initial PCH 2/17 Initial CB atrophy 6/17 Initial CBL atrophy 6/17 Progressive PCH 10/15 [8/15*] Progressive CB atrophy: 15/15 Progressive CBL atrophy: 14/15 N Pons & BS on last image 3/15 [5/15*] Lactate peak 4/6

F, female; M, male; NA, not available; ND, not described; CS, consanguineous; SGA, small for gestational age; CoH, Compound Heterozygote; hr, hours; d days; wk weeks; mo, months; y, years; SZ, seizures; SE, status epilepticus; EOEE, early-onset epileptic encephalopathy; EEG, electroencephalogram;

OFC, occipito-frontal circumference (according to Nellhaus, 1968 or as stated by authors); SD, standard deviation; Mic, microcephaly; QP quadriplegia; GDD, global developmental delay; VEP, Visual evoked potentials; ERG electroretinography; N Normal; CVI, Central visual impairment; GERD, gastro-esophageal reflux disease; CSF, cerebrospinal fluid; mod, moderate; MRC, mitochondrial Respiratory Complexes;

MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; CT, computerized tomography; CB, cerebral; CBL, cerebellar; CC, corpus callosum; BS, brainstem; SAS, subarachnoid spaces; PCH, pontocerebellar hypoplasia; WM, white matter

- Reviewing the figures provided by Cassandrini D et al 2013 we consider the pons of patient B-01 of normal volume

Figure(s)

A

B

C