

Expanding spectrum of *RARS2* gene disorders: Myoclonic epilepsy, mental retardation, spasticity, and extrapyramidal features

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SUMMARY

Pontocerebellar hypoplasia type 6 (PCH6) is an autosomal recessive mitochondrial disease, typically characterized by pontine atrophy, vermian hypoplasia, infantile encephalopathy, generalized hypotonia, and intractable seizures. The purpose of this study is to describe the seizures and other neurological manifestations of *RARS2* gene mutations and to compare the clinical features with other causes of progressive myoclonic epilepsy. Detailed history, physical examination, and clinical and genetic work-up were performed in 2 siblings who presented with progressive myoclonic epilepsy. One sibling, a 20-year-old woman, and the other a 24-year-old man, had a homozygous missense variant (c.848T>A; p.Leu283Gln) in exon 10 of the *RARS2* gene. The female patient had action and audiogenic myoclonic jerks, postural tremors, spastic dysarthria, and bradykinesia, and her male sibling had similar features with oculomotor apraxia. The *RARS2* gene mutation can present with myoclonic epilepsy, mental retardation, and pyramidal and extrapyramidal features, and is an important differential for causes of progressive myoclonic epilepsy.

KEY WORDS: Extrapyramidal features, Mental retardation, Myoclonic epilepsy, *RARS2* gene, Spasticity.

Pontocerebellar hypoplasia type 6 (PCH6), is an autosomal recessive mitochondrial disease caused by mutations in the *RARS2* gene.¹ The *RARS2* gene encodes mitochondrial arginyl transfer RNA synthetase, an enzyme necessary for mitochondrial protein translation.² PCH6 is typically characterized by pontine atrophy, vermian hypoplasia, infantile encephalopathy, generalized hypotonia, and intractable seizures.³ Herein we describe 2 siblings with novel

homozygous missense mutations of the *RARS2* gene that presented with myoclonic epilepsy, mental sub-normality, oculomotor apraxia, spasticity, and dystonia without pontine or vermian hypoplasia.

METHODS

Both siblings were born of a second-degree consanguineous marriage (Figure 1). A detailed history was obtained and a routine physical examination was conducted. Extensive clinical and biochemical workup was performed, including tandem mass spectroscopy (TMS), and serum lactate and ammonia tests. Spasticity was graded using the modified Ashworth scale. Brain magnetic resonance imaging (MRI) and electroencephalography (EEG) were also performed. Next-generation sequencing was carried out for genetic analysis. The complete list of clinical features of both siblings is summarized in Table 1.

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KEY POINTS

- *RARS2* gene mutation can result in myoclonic epilepsy, mental subnormality, spasticity, and extrapyramidal features like bradykinesia and dystonia
- *RARS2* gene-related myoclonic epilepsy is an important differential for causes of progressive myoclonic epilepsy
- In all cases of infantile onset myoclonic epilepsy *RARS2* gene mutation should be tested

Genetics

Whole-exome sequencing

DNA from the proband (Patient 1) was extracted from blood using standard protocol. Sequencing was performed on Illumina HiSeq 2500 using 100x2 V4 kit chemistry with an average sequencing depth of about 100X. Human reference genome GRCh37 was used for alignment. Genome Analysis Tool Kit was used for variant calling. International Haplotype Map Project, 1000 Genomes Project, Online Mendelian Inheritance in Man, data base for non synonymous single-nucleotide polymorphisms functional prediction, Polymorphism Phenotyping, Catalogue Of Somatic Mutations In Cancer, and Clinically important Variants

database were used for variant filtration and annotation. Further classification, annotation, and clinical interpretation was carried out using InterpretOmics database developed by manually curating clinical grade variant information from PubMed and other published data sources.

Sanger sequencing

High-quality DNA was extracted from the blood of the proband, her sibling, and parents using standard protocol. Primers were designed for the identified variant. DNA was then subjected to bidirectional Sanger sequencing to screen the variants in *RARS2* gene using ABI Prism 3730 Genetic Analyzer. Human reference genome GRCh38 was used for alignment.

RESULTS

Patient 1

A 20-year-old woman had an uneventful full-term birth; however, myoclonic jerks were noted immediately after birth. The patient had a history of multiple attacks of simple febrile seizures during childhood. Motor and mental milestones were delayed. The patient currently has mild mental sub-normality but is independent in activities of daily living.

On physical examination, the patient was found to have spastic dysarthria, postural tremors, bradykinesia,

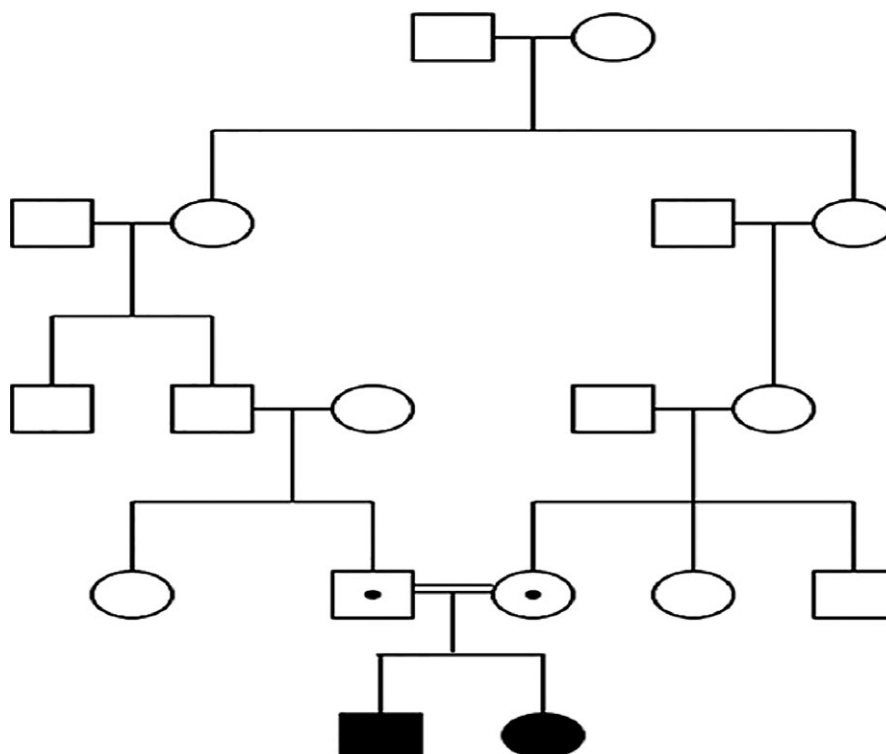


Figure 1.

Pedigree chart of the family showing the affected siblings and unaffected parents in a second-degree consanguineous marriage
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Table 1. Clinical features exhibited by the 2 siblings		
	Sibling 1	Sibling 2
Age/sex	24 year/Male	20 year/Female
Birth	Delayed cry	Uneventful
Myoclonus	Since birth Truncal and appendicular Action and sound sensitive	Since birth Truncal and appendicular Action myoclonus
	Improved with drugs	Poor response
	Falls due to jerks	No falls
Delayed milestones	Present	Present
Febrile seizures	Present	Present
Subnormal Intelligence	Severe	Moderate
Activities of daily living	Dependent	Independent
Oculomotor apraxia	Present	Absent
Spasticity	Present	Present
Ashworth score	2	2
Spastic dysarthria	Present	Present
Dystonia	Bradykinesia/dystonia	Mild
Tremors	Postural	Postural - mild
Gait	Spastic	Spastic
Medications	Valproate, topiramate	Valproate, lamotrigine, zonisamide
Fundus	No optic atrophy, retinitis pigmentosa, cherry red spot	
Hearing	Normal	

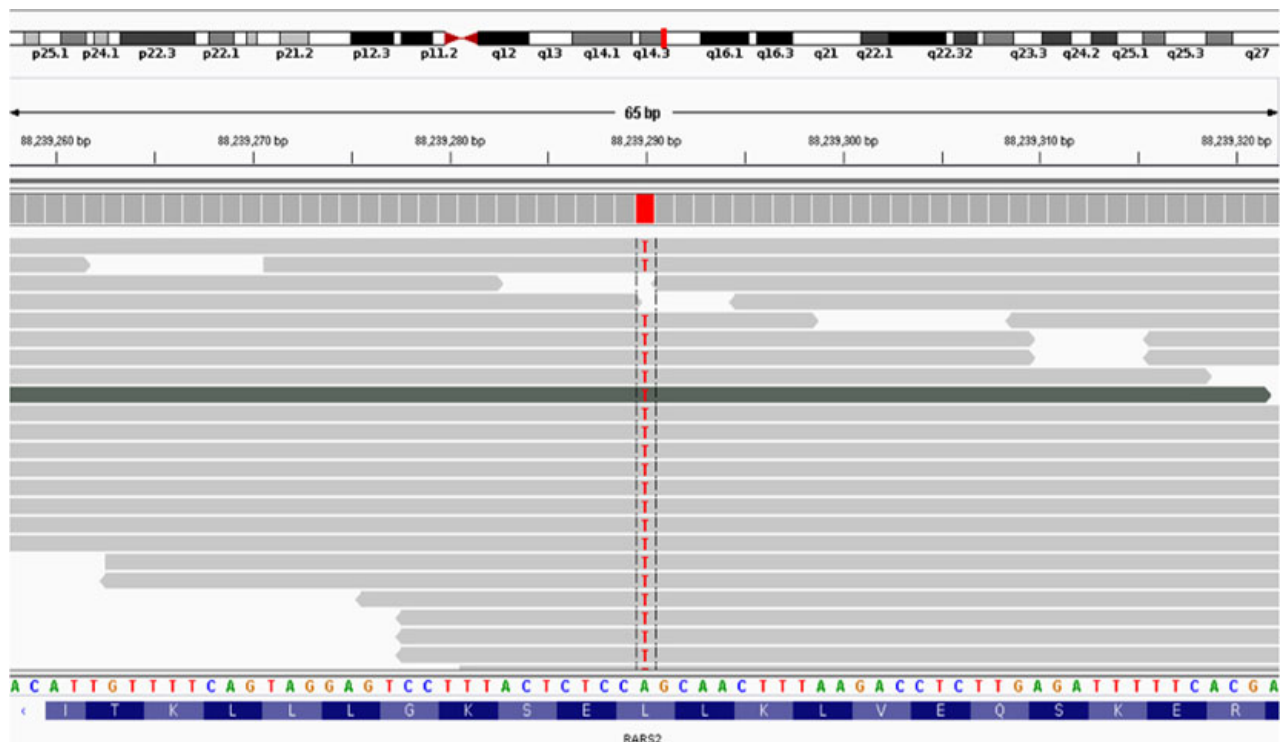


Figure 2.

Presence of RARS2 mutation is seen across all reads of sequencing in an Integrated Genome Viewer

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spasticity, and an axial and appendicular myoclonus precipitated by movement and sound (action and audiogenic). Deep tendon reflexes were brisk on both sides with extensor

plantar response. MRI of the brain was normal. EEG showed diffused slowing. TMS, and serum lactate and ammonia levels were normal. The patient was treated with

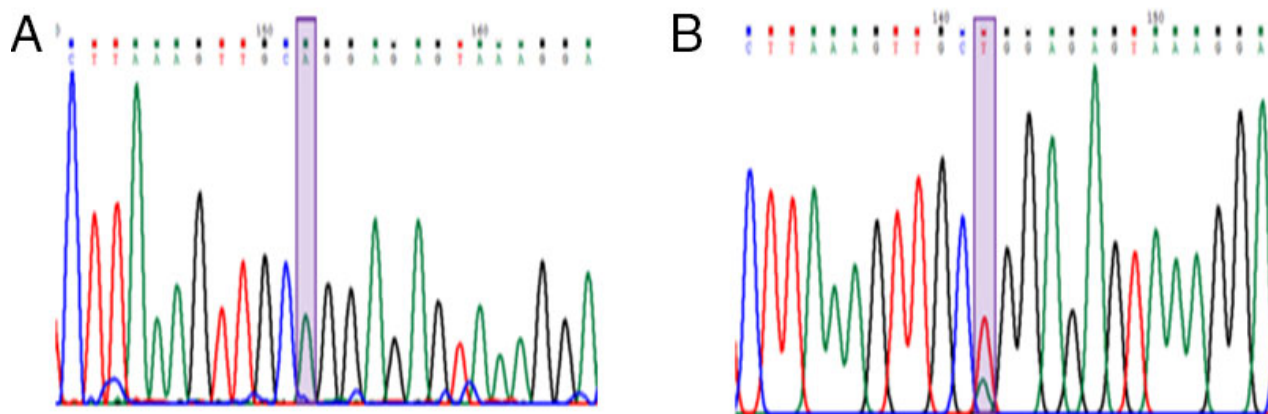


Figure 3.

Representative image of electropherograms obtained on Sanger evaluation. 3A depicts a homozygous ($T>A$) variant in the *RARS2* gene noted both in the proband and the affected sibling. 3B depicts the heterozygous form of the same variant that was identified in both parents

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valproate, levetiracetam, and topiramate previously, but myoclonic jerks were poorly controlled.

Patient 2

A 24-year-old man, brother of Patient 1, had myoclonic jerks involving all 4 limbs detected immediately after birth. The patient had a history of multiple attacks of simple febrile seizures during childhood. All developmental milestones were delayed. The patient had severe mental subnormality, never attended school, and was dependent in all activities of daily living.

On examination, the patient was found to have oculomotor apraxia, spastic dysarthria, action and audiogenic myoclonus, postural tremors, bradykinesia, dystonia of the limbs, and spasticity in all 4 limbs. Deep tendon reflexes were brisk on both sides with extensor plantar response.

MRI brain was normal. EEG showed diffused slowing. TMS and serum lactate and ammonia levels were normal. The patient was treated with sodium valproate and topiramate, and myoclonic jerks were fairly controlled.

Whole exome sequencing of the proband revealed a homozygous missense variant (c.848T>A; p.Leu283Gln) in exon 10 of the *RARS2* gene, visualized on an integrated genomic viewer. The variant was noted across all reads of the sequencing run confirming homozygosity (Figure 2). In silico analysis models (Mutation Taster, Polymorphism Phenotyping v2, and Sorting Intolerant From Tolerant) predict this variant to be pathogenic. This missense variant was not noted previously in the 1000 Genomes Project. Orthogonal validation of the identified variant was performed using Sanger sequencing, and the presence of the variant was confirmed in a homozygous

fashion. The unaffected parents and the affected sibling were also noted to have a heterozygous and homozygous copy of the variant, respectively, thus establishing a disease-variant segregation in the current family (Figure 3).

DISCUSSION

RARS2 mutations were first described in 2007 in a Jewish family with severe infantile encephalopathy.⁴ Typically, mutations of *RARS2* are characterized by neonatal lactic acidosis, intractable seizures, encephalopathy, hypotonia, spasticity, microcephaly, and profound developmental delay.³ Patients were found to have compound heterozygous mutations in a majority of the reported cases, whereas homozygous mutations have been reported in only 3 other families, apart from this case report.^{4–6} This missense variant has been reported previously in a compound heterozygous fashion in association with a 3 base-pair deletion in siblings affected with PCH6.⁷

To the best of our knowledge, there have been 29 cases of PCH6 reported from 15 families.¹ Compared to patients with classical PCH6, our patients had a milder phenotype and survived well into adulthood. Furthermore, no abnormalities such as pontine or vermian hypoplasia were detected through neuroimaging. Action and audiogenic myoclonus, postural tremors, oculomotor apraxia, spasticity, and dystonia were atypical features in these patients that have not been previously reported in PCH6. The association of myoclonic epilepsy with a *RARS2* mutation is novel and has not been reported previously. The features of *RARS2*-associated myoclonic epilepsy and other progressive myoclonic epilepsies have been compared in Table 2. Infantile onset may be an important clue to the diagnosis of *RARS2*-associated myoclonic epilepsy.

Table 2. Comparison of myoclonic epilepsy due to RARS2 mutation with other progressive myoclonic epilepsies⁸

	Age of onset (Vein)	Inheritance	Prominent seizures	Cerebellar signs	Dementia	Fundus	Dysmorphism	EEG	MRI	Other features
RARS2	Birth	AR	<ul style="list-style-type: none"> • Myoclonus • Febrile seizures 	–	Mental retardation	Normal	No	Diffuse slowing	Normal	Oculomotor apraxia, EPS, spasticity and mental retardation Most common PME
Unverricht-Lundborg disease	6–15	AR	Stimulus-sensitive myoclonus GTCS	Mid and late	Mild and late/absent	Normal	No	Generalized spike-wave, poly-spike discharges with photosensitivity	Normal	
Lafora disease	12–17	AR	<ul style="list-style-type: none"> • Stimulus sensitive myoclonus • Occipital seizures 	Early	Early and relentless	Normal	No	Disorganized with high-voltage spike-wave discharges	Diffuse cortical atrophy	Early emotional disturbance death within 10 years of onset
Myoclonic epilepsy with ragged red fibers	Any age	Mitochondrial	Myoclonus	Variable	Variable	Optic atrophy/retinopathy +/-	+/-	Generalized spike wave discharges at 2-5 Hz	Atrophy with basal ganglion calcification	Ragged red fibers in muscle biopsy, neuropathy, hearing loss
Neuronal ceroid Lipofuscinosis	Variable	AR/AD	Variable	Variable	Rapidly progressive	Macular degeneration and visual failure	No	Background disorganization with generalized epileptiform discharges	Atrophy	5 types, psychosis, hallucinations, early death and visual loss may be seen
Sialidosis	Variable	AR	Intention and action myoclonus	Second or third decade	Type I-absent Type II – learning difficulty	Cherry red spot	Type II ++	Low-voltage fast activity	Atrophy in late stages	Hepatosplenomegaly, recurrent respiratory infections

AD, autosomal dominant; AR, autosomal recessive.

CONCLUSION

This case report expands on the clinical spectrum of *RARS2* mutations to include myoclonic epilepsy, spasticity, bradykinesia, dystonia, and oculomotor apraxia, in addition to the classical features. In patients of myoclonic epilepsy with atypical features, clinicians should test for *RARS2* mutations.

DISCLOSURE

We have no conflict of interest to declare. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines

FINANCIAL DISCLOSURE

None.

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