HHS Public Access

Author manuscript

Clin Genet. Author manuscript; available in PMC 2023 March 07.

Published in final edited form as:

Clin Genet. 2021 April ; 99(4): 594–600. doi:10.1111/cge.13928.

Bi-allelic missense variant, p.Ser35Leu in *EXOSC1* is associated with pontocerebellar hypoplasia

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Abstract

RNA exosome is a highly conserved ribonuclease complex essential for RNA processing and degradation. Bi-allelic variants in exosome subunits *EXOSC3, EXOSC8* and *EXOSC9* have been reported to cause pontocerebellar hypoplasia type 1B, type 1C and type 1D, respectively, while those in *EXOSC2* cause short stature, hearing loss, retinitis pigmentosa and distinctive facies. We ascertained an 8-months-old male with developmental delay, microcephaly, subtle dysmorphism and hypotonia. Pontocerebellar hypoplasia and delayed myelination were noted on neuroimaging. A similarly affected elder sibling succumbed at the age of 4-years 6-months. Chromosomal microarray returned normal results. Exome sequencing revealed a homozygous missense variant, c.104C > T p.(Ser35Leu) in *EXOSC1* (NM_016046.5) as the possible candidate. *In silico* mutagenesis revealed loss of a polar contact with neighboring Leu37 residue. Quantitative real-time PCR indicated no appreciable differences in *EXOSC1* transcript levels. Immunoblotting and blue native PAGE revealed reduction in the EXOSC1 protein levels and EXO9 complex in the proband, respectively. We herein report an individual with the bi-allelic variant c.104C>T p.(Ser35Leu) in *EXOSC1* and clinical features of pontocerebellar hypoplasia type 1. Immunoblotting and blue native PAGE provide evidence for the pathogenicity of the variant. Thus, we propose *EXOSC1* as a novel candidate gene for pontocerebellar hypoplasia.

1 INTRODUCTION

The RNA exosome is an evolutionarily conserved and ubiquitously expressed ribonuclease complex. It is essential for the surveillance, processing, and degradation of various classes of RNA in the nucleus and cytoplasm with 3′- 5′ exoribonuclease and endonuclease

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

activity[1-3]. The cytoplasmic exosome complex consists of 10 subunits. Six of these proteins, EXOSC4 to EXOSC9, harboring a “RNase PH-like domain”, form the barrel-shaped central core. Three proteins, EXOSC1, EXOSC2 and EXOSC3, form the cap and aid in stable assembly of the core. These subunits consist of an N-terminal domain and S1-KH RNA-binding domains[4, 5]. These nine subunits (collectively called EXO9) are catalytically inactive and form the structural component of RNA exosomes. DIS3 (EXOSC11), forms the catalytic component of the complex and associates at the base of the central core. RNAs are guided down through the EXO9 core to the DIS3 for processing[4, 6-8].

Pathogenic variants in exosome complex proteins, EXOSC3, EXOSC8, EXOSC9, EXOSC2 and its cofactors, RBM7, SKIV2L, TTC37 are reported to cause human diseases[9-15]. Three of the four subunits complex proteins, (EXOSC3, EXOSC8 and EXOSC9) are associated with subtypes of pontocerebellar hypoplasia type 1[9, 10, 15]. Defects in the fourth subunit, encoded by *EXOSC2*, cause a distinct syndrome of short stature, hearing loss, retinitis pigmentosa and distinctive facies (MIM# 617763). However, progressive cerebellar atrophy is a component of this syndrome as well[11]. In this study, we report a child with developmental delay, microcephaly, facial dysmorphism, short stature, hypotonia, pontocerebellar hypoplasia and delayed myelination due to a missense variant, c.104C > T in *EXOSC1*.

2 MATERIALS AND METHODS

2.1 Clinical report

We ascertained an 8-months-old male with developmental delay. He is the second born to a third-degree consanguineous couple (Figure 1A,B). On examination, blue sclera, tall forehead, telecanthus, strabismus, depressed nasal bridge, anteverted nares, thick vermillion border of lips, long and smooth philtrum and retrognathia were noted (Figure 1B). Magnetic resonance imaging (MRI) of the brain at 8 months of age showed hypoplastic cerebellum with mild dilatation of folia, vermian hypoplasia, relative sparing of pons, prominent prepontine and cerebellopontine angle cisterns. A prominent cerebrospinal fluid intensity was noted in the posterior fossa with mild elevation of the cerebellum, likely to be mega cisterna magna. Thinning of corpus callosum and mild delay in myelination was noted (Figure 1C). There is also prominent extra-axial fluid space in the frontotemporal region suggestive of associated cerebral atrophy. Detailed clinical findings are provided in the supplementary information.

2.2 Genetic analysis and functional validation

Details of genetic testing, in silico protein modeling, cell culture, RNA extraction and RT-PCR, quantitative real-time PCR (qRT-PCR), immunoblotting, Blue native PAGE are provided in the supplementary information.

3 RESULTS

3.1 Variant identification

Chromosomal microarray returned normal results. Exome sequencing revealed a missense variant, c.104C>T p.Ser35Lue in the exon 2 of *EXOSC1* (NM_016046.5) in homozygous state as the possible candidate. His parents were found to be heterozygous carriers of the variant (Figure 1D). This variant was not observed in gnomAD and our in-house exome data of 960 individuals. The variant lies in the N-terminal RPL27-like domain of EXOSC1 protein. The amino acid at this position is highly conserved across mammals (GERP_RS score: 5.44). *In silico* tools such as MutationTaster, Polyphen-2, SIFT, M-CAP, CADD, FATHMM_MKL and REVEL predicted the variant to be damaging to the protein function.

3.2 Functional validation

Upon sequence homology-based protein modeling of EXOSC1, the wild-type amino acid residue, Ser35 was noted to have a single polar contact with neighboring residue Leu37. *In silico* mutagenesis of wild-type Ser35 to mutant Leu35 resulted in loss of the said polar interaction. This is likely to result in altered secondary protein structure of EXOSC1 (Figure 1F). RT-PCR detected no abnormal splicing due to the presence of the variant, c.104C>T in *EXOSC1*, and qRT-PCR showed no change in the expression of *ESOXC1* mRNA in the proband when compared to two unrelated healthy controls (Supplementary Figure 1). Immunoblotting performed on cultured fibroblasts lysate to detect the expression of RNA exosome complex proteins, EXOSC1, EXOSC3 and EXOSC8 revealed a significant reduction in the EXOSC1 protein in the proband compared with two healthy controls. EXOSC3 and EXOSC8 protein levels remained unaltered (Figure 2A). BN-PAGE performed using protein lysate from the fibroblasts of proband and two healthy controls revealed the significant reduction of the exosome complex in the proband (Figure 2B).

4 DISCUSSION

The proband presented with clinical features consistent with PCH type 1 and a bi-allelic missense variant, c.104C>T in *EXOSC1*. The phenotype is similar to defects in the closely related proteins of the exosome complex. Immunoblotting and blue native PAGE experiments with patient's fibroblast provide functional evidence for the pathogenicity of the variant.

Individuals with pathogenic variants in *EXOSC3, EXOSC8* and *EXOSC9* present with developmental delay, failure to thrive, recurrent pulmonary infections, hypotonia progressing to spasticity, cerebellar signs like nystagmus and tremors, subtle facial dysmorphism with or without seizures and early demise. Muscle weakness secondary to central and peripheral motor dysfunction has been noted in all PCH type 1 subtypes. Nerve conduction studies showed presence of axonal neuropathy and muscle biopsy revealed the findings of spinal muscular atrophy (SMA) like muscle atrophy in most. Although the proband has significant axial and peripheral hypotonia, these tests could not be performed. Regression of attained milestones have been noted to occur post seizure episodes. Of note, blue sclera was noted in the current proband and individuals affected with *EXOSC9*-related PCH. Other systemic

findings include hearing and vision impairment, oculomotor apraxia, retinal dystrophy, cholelithiasis and tongue atrophy and fasciculations, hyperthyroidism, glaucoma, retinitis pigmentosa, corneal dystrophy and atrial hypertension[9, 10, 15]. MRI brain in the present proband showed hypoplasia of cerebellum and vermis with relative sparing of pons, thinning of corpus callosum and delayed myelination. These findings are similar to those observed in PCH type 1[16]. Phenotypic and genotypic comparison of exosome complex related disorders are provided in the Table 1.

EXOSC1 encodes a structural component of the RNA exosome complex that is involved in the general processing and degradation of coding and non-coding RNAs[4]. The variant identified in this study, p.Ser35Lue is present in the highly conserved N-terminal RPL27-like domain of EXOSC1. Transcript analysis followed by immunoblotting in the patient fibroblast revealed that even with the normal *EXOSC1* mRNA expression the protein was significantly reduced. This finding suggests that change in the amino acid from serine to leucine at p.35 might affect the stability of the EXOSC1 protein. Previously, missense variants in different exosome subunits were shown to significantly reduce the amount of protein[9, 10, 17]. Each subunit of the complex is critical for its assembly and defects in any one subunit might affect the complex formation[10]. Previous studies in individuals harboring missense variants in three exosome subunits, showed a consequent reduction in the exosome complex assembled[9, 10, 17]. Similarly we observed that the EXOSC1 variant (p.Ser35Leu) resulted in a decrease in the exosome complex detected in proband fibroblasts, although this was less marked compared to that seen previously in EXOSC3 and EXOSC8 mutant primary cells. This supports the notion that the primary reduction in the levels of any one component of the exosome destabilizes the complex assembly.

Studies using model organisms have revealed the critical role of the exosome complex in brain development. Knockdown experiments of *exosc9* in zebrafish, showed defect in development of cerebellum and hindbrain hypoplasia, and motor neurons migration[10]. Poor motility and small brain size were observed in exosc3 knockdown zebrafish models[15]. Downregulation exosc8 in zebrafish showed abnormal midbrain, hindbrain and impaired myelination[9]. Cerebellar atrophy and microcephaly were observed in exosc8 and exosc9 mutant zebrafish with significant increase in the transcript levels of p53. Increased p53 correlates with increased number of brain cells undergoing apoptosis during development[17]. These findings reflect phenotypes observed in the patients with variants in genes coding for exosome subunits and the importance of exosome complex in brain development.

In this study, we propose *EXOSC1* as a novel candidate gene for pontocerebellar hypoplasia type 1. Report of additional individuals and further studies in model organisms would confirm *EXOSC1* as a cause of pontocerebellar hypoplasia *EXOSC1* related pontocerebellar hypoplasia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We are grateful to patient and his family for participating in the study. We thank Dr Govindaraj Periyasamy, Institute of Bioinformatics, Bengaluru and Deepha Sekar, Department of Neuropathology, NIMHANS, Bengaluru for their valuable suggestions in performing BN-PAGE experiment. This work was funded by Department of Health Research, Ministry of Health and Family Welfare, Government of India under the project titled “Clinical and Molecular Characterization of Leukodystrophies in Indian Children” grant ID—V.25011/379/2015-GIA/HR and National Institutes of Health, USA under the project titled ‘Genetic Diagnosis of Neurodevelopmental Disorders in India’ Grant ID—R01 HD093570 01 A1 (PI).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from corresponding author upon reasonable request.

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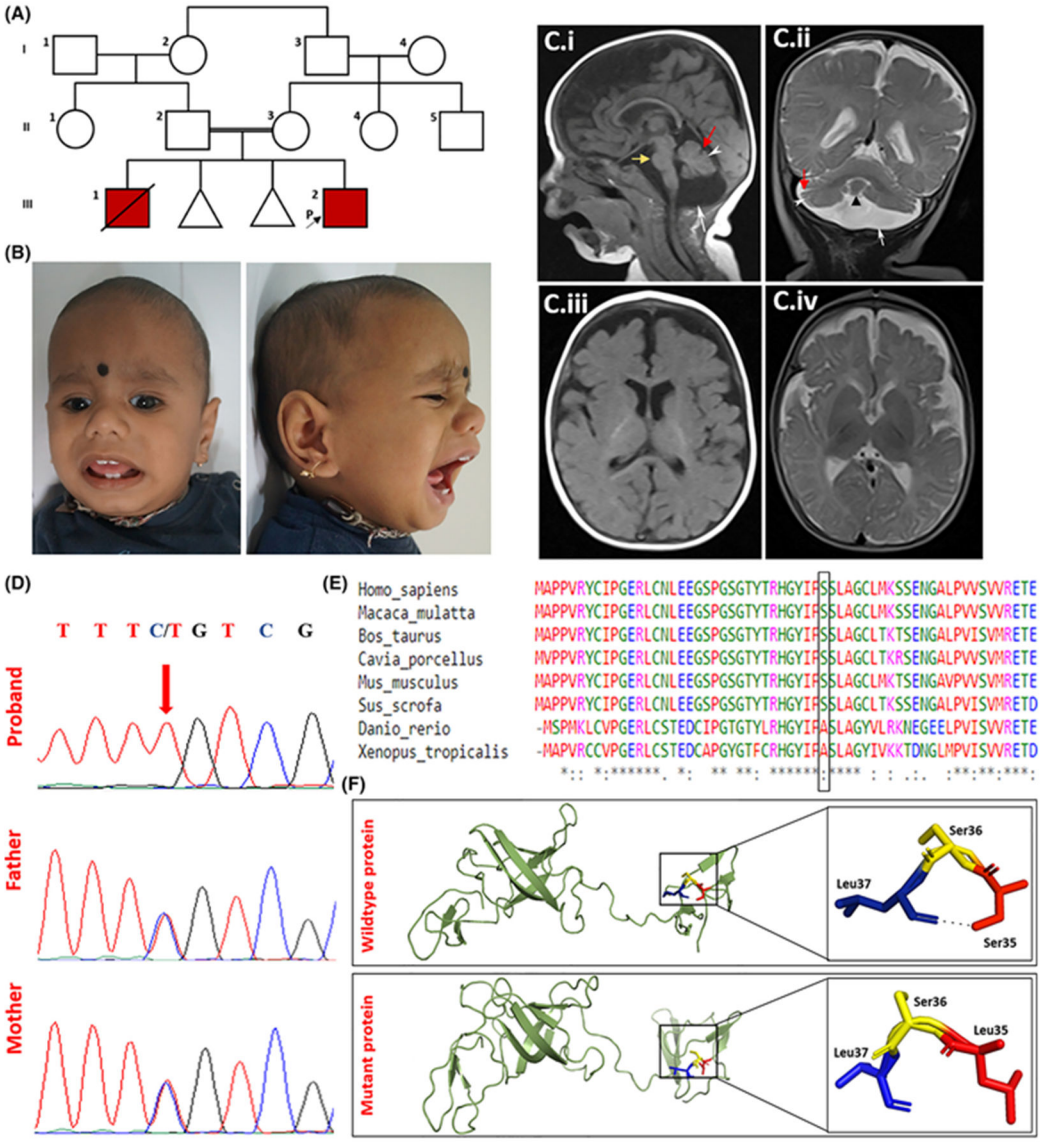

FIGURE 1.

Clinical, radiological, molecular and *in silico* findings in the proband. A, Pedigree of the family. B, Clinical photographs of the proband. C, MRI of the brain at 8 months shows cerebellar hypoplasia (white arrowhead), mega cisterna magna (white arrow), mild dilatation of cerebellar folia (red arrow), vermian hypoplasia (black arrowhead), relatively preserved pons (yellow arrow) (i and ii), mild hypoplasia of corpus callosum. (i) and mild delayed myelination according to age (iii and iv). Extra-axial CSF spaces in bilateral frontotemporal region appear prominent (iv) suggestive of associated cerebral atrophy. D, Electropherograms of the sequence variant, c.104C > T in EXOSC1 in the proband (homozygous) and parents (heterozygous). E, Multiple alignment of EXOSC1 sequences across multiple species (Ser35 highlighted). F, Protein structure prediction shows replacement of the Ser35 with mutant Leu35 is predicted to lead to loss of a polar contact resulting in altered stability of the protein.

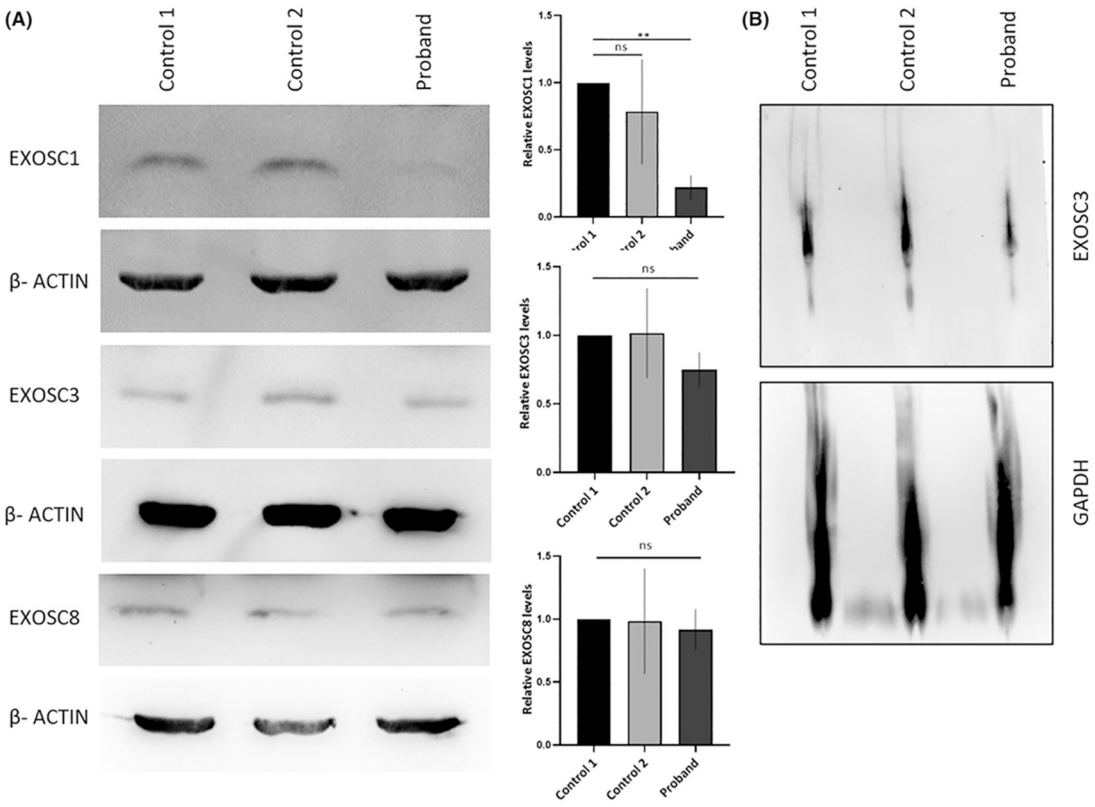

FIGURE 2.

Immunoblotting and BN-PAGE determine reduced levels of EXOSC1 and exosome complex. Immunoblotting of RNA exosome complex proteins, EXOSC1, EXOSC3 and EXOSC8 in patient and controls. Significant reduction in EXOSC1 was observed in the proband compared to two unrelated healthy controls. EXOSC3 and EXOSC8 levels remained unaltered in the patient. β-Actin was used as loading control. Results are the mean ± SEM of at least three different experiments (**$P < .05$, One-way ANOVA followed by Dunnett's multiple comparison test). B, BN-PAGE revealed a reduction of the assembly of the exosome complex in the proband's fibroblast. GAPDH was used as a loading control.

TABLE 1.

Phenotypic and genotypic comparison of exosome complex-related disorders

Gene	***EXOSC1***	***EXOSC8***	***EXOSC9***	***EXOSC3***	***EXOSC2***
Disorder	Pontocerebellar hypoplasia (proband)	Pontocereblellar hypoplasia type 1C	Pontocereblellar hypoplasia type 1D	Pontocerebellar hypoplasia, type 1B	Short stature, hearing loss, retinitis pigmentosa and distinctive facies
Number of families (individuals)	Not applicable	3 (22)	7 (7)	58 (82)	2 (3)
Type of sequence variant	Missense	Missense	Missense, stopgain in trans with missense	Missense, frameshift deletion, splicing variant	Missense
Clinical findings					
Age of onset	Infancy	Infancy	Intrauterine life to infancy	Infancy	Variable (birth to third decade)
Developmental delay	Yes	Yes	Yes	Yes	Yes
Feeding difficulties	No	No	Yes (in few)	Yes (in few)	No
Seizures	No	No	Yes (in two related individuals)	Yes (in few)	No
Growth retardation	Yes	Yes	Yes	Yes	Yes
Facial dysmorphism	Yes	Yes (in some)	Yes (in some)	Yes	Yes
Microcephaly	Yes	Yes	Yes	Yes	Yes
Tone	Hypotonia	Spastic tetraparesis	Severe hypotonia of limbs	Axial hypotonia to spasticity	NA
Deep tendon reflexes	Diminished	Diminished/ exaggerated	Absent	Diminished (in few)	NA
MRI findings					
Cerebellar signs	No	Tremors	Nystagmus	Intention tremors, nystagmus	Nystagmus
Joint contractures	No	Yes	Yes (arthrogryposis multiplex congenita)	Yes	NA
Cerebellar hemisphere hypoplasia	Yes	Yes	Yes	Yes	Yes
Cerebellar vermian hypoplasia	Yes	Yes	Yes	Yes	No
Dragonfly appearance	No	No	No	Yes (in few)	No
Dilatation of cerebellar folia	Yes	No	No	No	No
Cerebellar cysts	No	No	No	Yes	No
Pontine hypoplasia	Yes	Yes (in few)	Yes (in few)	Yes (in few)	No
Cerebral atrophy	Yes	Yes	Yes (in few)	Yes	No
Corpus callosum abnormalities	Thinning of corpus callosum	Thinning of corpus callosum	No	No	No
Myelination abnormalities	No	Yes	No	No	Yes
Other investigations					

Gene	***EXOSC1***	***EXOSC8***	***EXOSC9***	***EXOSC3***	***EXOSC2***
Muscle biopsy	Not done	SMA-like findings	SMA-like findings	SMA-like findings	NA
NCV/EMG	Not done	Motor neuronopathy	Axonal motor neuronopathy	Neurogenic changes on EMG	NA
Ophthalmology evaluation	Blue sclera	Impaired vision	Blue sclera, absent fixation, impaired pursuit	Oculomotor apraxia, poor visual attention, nystagmus, strabismus, retinal dystrophy	Retinitis pigmentosa, corneal dystrophy, glaucoma, nystagmus, strabismus
Hearing evaluation	Not done	SNHL	Normal (available for four probands)	Not available	SNHL

Abbreviations: EMG, electromyography; MRI, magnetic resonance imaging; NCV, nerve conduction velocity study; NA, not available; SMA, spinal muscular atrophy; SNHL, sensory neural hearing loss.