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SHORT COMMUNICATION

# Exome sequencing in a family with intellectual disability, early onset spasticity, and cerebellar atrophy detects a novel mutation in *EXOSC3*

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**Abstract** Whole exome sequencing in two-generational kindred from Bangladesh with early onset spasticity, mild intellectual disability, distal amyotrophy, and cerebellar atrophy transmitted as an autosomal recessive trait identified the following two missense mutations in the *EXOSC3* gene: a novel p.V80F mutation and a known p.D132A change previously associated with mild variants of pontocerebellar hypoplasia type 1. This study confirms the involvement of RNA processing proteins in disorders with motor neuron and cerebellar degeneration overlapping with spinocerebellar ataxia 36 and rare forms of hereditary spastic paraplegia with cerebellar features.

**Keywords** Exosome component 3 (EXOSC3) · Hereditary spastic paraplegia (HSP) · Pontocerebellar hypoplasia type 1 (PCH1) · Spinocerebellar ataxia type 36 (SCA36) · Whole exome sequencing (WES)

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## Introduction

Homozygous or compound heterozygous mutations in human exosome component 3 (*EXOSC3*), also known as the ribosomal RNA–processing protein 40 (RRP40), a noncatalytic subunit of the exosome complex involved in the processing and turnover of several RNA species, have recently been related to pontocerebellar hypoplasia type 1 (PCH1), a severe progressive condition that associates prenatal or congenital onset hypotonia, oculomotor dysfunction, signs of respiratory failure leading to death within the first years of life together with progressive microcephaly, severe and global developmental delay, and anterior horn cell disease [1]. We report on the genetic and phenotypic analysis of two-generational kindred from Bangladesh with an autosomal recessive form of early onset spasticity associated with mild cognitive impairment, cerebellar atrophy, and distal amyotrophy carrying *EXOSC3* mutations.

## Subjects and methods

### Subjects

The family originates from Bangladesh and immigrated in Italy. Parents are unrelated and healthy. The two affected sibs, a male and a female, had similar neurological symptoms. Muscular biopsy, electrophysiological studies, and brain MRI were performed using standard protocols. All examined family members gave written consent, and the study fulfilled our institution's ethical rules for human genetic studies.

#### *Patient 1*

The boy, now aged 19 years, was born at term after an uneventful delivery and appeared healthy. Markedly delayed

motor milestones were first noticed at 1 year of life; the child was able to sit alone at age 5 and walked unassisted between age 12 and 14 but then lost this ability for worsening of the spasticity and became wheelchair bound. The neurological examination showed bilateral adducted thumbs, talipes valgus, tongue atrophy, and fasciculations (Fig. 1a, c, e, h). Head circumference was normal (54 cm). Tendon jerks were brisk, and he had horizontal gaze-evoked nystagmus. He had a moderate intellectual disability with preserved language comprehension; he was able to speak in sentences with a severe dysarthria, interacted with a computer and eats with no aid. The somatosensory-evoked potentials (SSEPs) recorded after stimulation of tibial and median nerves showed that only central conduction times were delayed. EMG showed normal nerve conduction velocity and a neurogenic pattern in all the explored muscles. A brain MRI performed at age 10 and showed a marked cerebellar atrophy with prominent sulci and decreased volume of folia, normal brainstem, and cortex (Fig. 1k–m). A muscle biopsy in the quadriceps femoris muscle showed neurogenic abnormalities with grouping limited to type II muscle fibers (Fig. 1o).

*Patient 2*

The affected sister is now 14 years old; she had delayed motor milestones and started to walk at age 3 years. She attends school and has a mild intellectual disability. She is able to walk with minimal support, and she speaks in sentences with mild dysarthria. OFC is normal (50 cm), and she has horizontal gaze-evoked nystagmus. Tendon jerks were brisk and bilateral adducted thumbs, talipes valgus, tongue atrophy, and fasciculations were present (Fig. 1b, d, f, i). The electrophysiological examination showed delayed central conduction times at SSEPs with EMG showing signs of neurogenic involvement at distal muscles. A brain MRI performed at age 7 showed in both sibs marked cerebellar atrophy with prominent sulci and decreased volume of folia, normal brainstem, and cortex (Fig. 1l–n).

## Exome sequencing

Exome sequencing was performed on the genomic DNA of the two affected individual and their normal parents using NimbleGen Sequence Capture technology (SeqCap EZ library) according to the manufacturer's instructions. The enriched libraries underwent 90 base pair paired-end sequencing on a HiSeq2000 next-generation sequencing platform (Illumina, San Diego, CA). The sequence data were aligned to the reference human genome (UCSC hg19) using SOAPaligner and variant calling used the SOAPsnp (v.1.03). After filtering PCR duplicates, previously reported variants (dbSNP135 as reported in the UCSC Genome Browser (http://genome.ucsc.edu/), the 1000 Genomes Project (http://www.1000genomes.org) and HapMap 8 (http://hapmap.ncbi.nlm.nih.gov/) databases, with

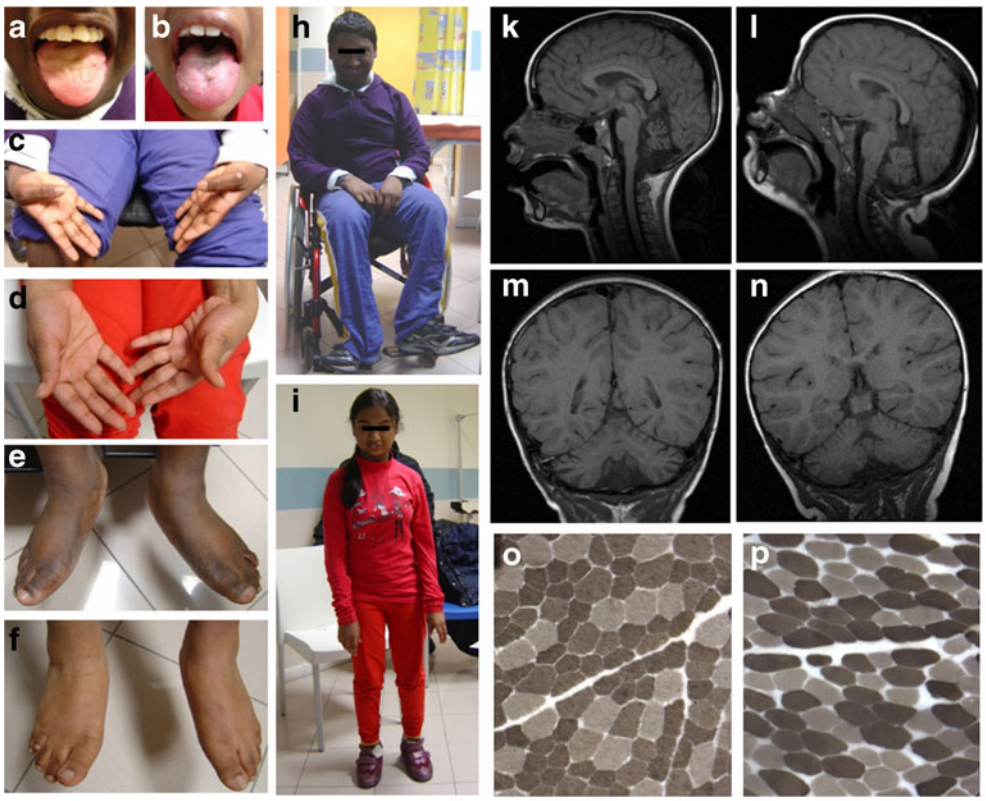


**Fig. 1** Clinical and morphological studies of affected sibs. Clinical pictures showing **a**, **b** tongue atrophy and fasciculations (**c**, **d**) adducted thumbs (**e**, **f**) talipes valgus feet (**h**, **i**) affected brother unable to walk and affected sister standing up without support. Brain MRI (**k**, **l**). Mid-sagittal T1-weighted image showing an overall size reduction of the cerebellum predominant in the vermis with widened interfolia sulci. Brainstem appears normal (**m**, **n**). Coronal T1-weighted image showing global cerebellar atrophy and normal cortex. **o**, **p** Muscle biopsy of the quadriceps femoris muscle (ATPase 9.4 staining) showing neurogenic abnormalities with grouping limited to type II muscle fibers in the patient (*left*) control muscle biopsy(*right*)

frequency greater than 0.5 %,) and synonymous variants were removed. For potential disease-causing variants, protein alteration was predicted using sorting intolerant from tolerant (SIFT; http://sift.bii.a-star.edu.sg/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) softwares. The variants were confirmed using Sanger sequencing of all four exons and flanking introns of *EXOSC3*, as previously described [1].

## Results

### Mutation analysis

Known genes or variants within the loci linked to spinocerebellar ataxia/spastic paraplegia were excluded by WES data analysis. Variants were prioritized according to the presence of compound heterozygosity based on recessive inheritance and on the probable non-consanguinity of the parents. We detected two missense mutations in the exosome subunit *EXOSC3* (GenBank accession no. NM_016042.3): c. 395A>C; pD132A (observed in 6 of 4,870 exomes, with an estimated allele frequency of 0.0012 and present in more than 50 % of PCH1 families [4], and a novel c. 238G>T; pV80F mutation( Fig. 2) in the conserved N-terminal domain of the protein, which was predicted to be deleterious by all prediction softwares.

## Discussion

Mutations in human *EXOSC3*, also known as the ribosomal RRP40, a noncatalytic subunit of the exosome complex involved in the processing and turnover of several RNA species, have recently been related to PCH1, a severe condition that associates prenatal or congenital onset hypotonia, oculomotor dysfunction, and signs of respiratory failure leading to death within the first years of life together with progressive microcephaly, severe and global developmental delay, and anterior horn cell disease. Neuroimaging generally shows cerebellar and pontine atrophy. PCH1 has more rarely been related to mutations in other RNA processing genes, *RARS2* (mitochondrial arginyl-tRNA synthetase 2) and *TSEN54* (tRNA splicing endonuclease 54) and in *VRK1* (vaccinia-related kinase 1) [2]. Very recently, the clinical spectrum related to mutations in *EXOSC3* was reported to be wider in a study of a large series of PCH1 cases including patients that appeared normal at birth but presented with psychomotor retardation within 6 months of life and had long survival [3]; however, the maximal functional ability of a single patient was the achievement to crawl and to perform a few steps with support. The neuroimaging spectrum of patients with *EXOSC3* mutations is also known to include cerebellar atrophy with relatively preserved brainstem and pons. The patients here reported present early-onset spasticity with intellectual disability, distal amyotrophy, and no progression or very slow worsening of neurological symptoms. Absence of hypotonia, microcephaly, and achievement of autonomous walking clearly distinguish it from PCH1, even the mildest variants related to homozygous D132A mutations (see supplemental Table S1). The common pD132A in association with other mutations has been associated to moderate or severe classical PCH1 phenotypes. The crystal structure of rrp40/EXOSC3 has extensively been explored and suggests that residue D132 may be important for intersubunit (rRP45/EXOSC9) interaction within the exosome complex and falls into the putative RNA binding S1 domain, whereas the V80 residue falls into the N-terminal domain of EXOSC3 with apparently no direct interaction with RNA

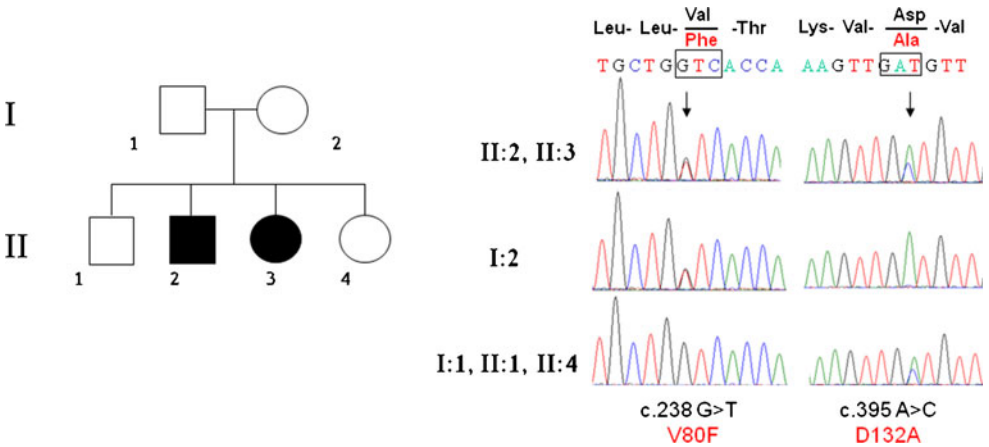


**Fig. 2** Pedigree structure of the family and electropherograms of the *EXOSC3* mutations. On the *left* side of the panel is the pedigree structure of the family; the *squares* and *circles* denote males and females, respectively. *Filled symbols* represent the two probands, and *open symbols* indicate unaffected individuals. On the right side of the panel are the electropherograms of the heterozygous *EXOSC3* mutations, c.395 A>C (p.Asp132Ala) and c.238G>T (p.Val80Phe) both present in affected individuals. The mother (I:2) is carrier of the V80F mutation, while the father (I:1) and the unaffected sibs (II:1, II:4) carry the D132A mutation. All family members except unaffected sibs underwent whole-exome sequencing

substrates or other exosome subunits, although close residues 81 and 82 interact with exosome subunit rrp46/EXOSC5 [4, 5]. The novel mutation V80F identified in the present family might be even less deleterious than pD132A mutations for EXOSC3 function.

The phenotype of the affected sibs partially overlaps with hereditary spastic paraplegias (HSP), a clinically variable and genetically heterogeneous group of neurodegenerative disorders of the motor system characterized by slowly progressive lower-limb spasticity, primarily caused by dysfunction or degeneration of upper motor neurons, and in particular with complex HSP forms with cerebellar features [6]. Additional features such as bilateral adducted thumbs are reminiscent of spastic paraplegia type 1. Although ataxia is not evident in the affected sibs, tongue fasciculations, dysarthria, cerebellar atrophy, and motor neuron degeneration share some clinical overlap with spinocerebellar ataxia 36, caused by mutations of NOP56, a factor involved in small nucleolar RNA maturation which binds to exosome-associated protein Rrp47 [7, 8].

Abnormalities of RNA metabolism are emerging as pathogenetic mechanisms of several motor neuron disorders [9]. Accumulation of aberrant tRNAs due to loss of a RNA kinase interacting with the TSEN complex sensitize motor neurons to oxidative stress-induced p53 [10]. Interestingly, yeast two-hybrid studies have identified the CDK5 repressor protein CDK5RAP1, a nuclear and mitochondrial tRNA processing protein, as EXOSC3 interactor [11]. CDK5 phosphorylates p53 and its dysregulation is involved in the development of various neurodegenerative diseases. The present study indicates that *EXOSC3* mutations can underlie clinical phenotype not classifiable as PCH1, and that screening of this gene should be implemented in cases of cerebellar atrophy combined with signs of motor neuron degeneration. This study again confirms WES as a powerful diagnostic tool for molecular testing [12] of highly genetically and clinically heterogeneous conditions, contributing to the expansion of the phenotypic spectrum of rare disorders by identifying known genes in novel phenotypes.

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