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Short communication

Investigating *PUM1* mutations in a Taiwanese cohort with cerebellar ataxia

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ABSTRACT

Introduction: Mutations in the *PUM1* gene were recently identified to cause spinocerebellar ataxia type 47 (SCA47). However, their role in cerebellar ataxia in various populations remains elusive. The aim of this study was to elucidate the frequency and spectrum of *PUM1* mutations in a cohort of Taiwanese patients with molecularly undetermined cerebellar ataxia.

Methods: Mutational analyses of *PUM1* were performed by Sanger sequencing in a cohort of 248 unrelated patients with cerebellar ataxia of unknown cause, including 108 with autosomal-dominantly inherited cerebellar ataxia, 45 with autosomal-recessively inherited cerebellar ataxia, and 95 with apparently sporadic cerebellar ataxia. Among them, the genetic causes of ataxia remained unknown after excluding mutations responsible for SCA1, 2, 3, 6, 7, 8, 10, 12, 17, 19/22, 23, 26, 27, 28, 31, 35, 36, dentatorubral-pallidoluysian atrophy and Friedreich's ataxia.

Results: Two heterozygous missense *PUM1* variants were identified in two patients with apparently sporadic cerebellar ataxia, including a known disease-causing mutation (p.R1139W) and a variant of uncertain significance (p.K151R). The patient carrying the p.R1139W mutation had a slowly progressive, relatively pure cerebellar ataxia, presenting with gait unsteadiness, limb dysmetria, ataxic dysarthria and saccadic pursuit.

Conclusion: Our findings support the pathogenic role of *PUM1* mutations in cerebellar ataxia and emphasize the importance of considering *PUM1* mutations as a possible etiology of cerebellar ataxia.

1. Introduction

The spinocerebellar ataxias (SCAs) encompass a group of hereditary neurodegenerative diseases characterized by progressive cerebellar degeneration with or without other neurological system involvement, exhibiting a wide range of clinical presentations, including unsteady gait, limb clumsiness, dysarthria, oculomotor abnormality, cognitive impairment, pyramidal and/or extrapyramidal signs [1]. Currently, 37 causative genes have been identified in 41 dominantly inherited SCA loci (SCA1-48) [1,2]. Most SCA cases are caused by an expansion or insertion of a trinucleotide or oligonucleotide repeat, including SCA1, 2, 3, 6, 7, 8, 10, 12, 17, 31, 36, and 37 [1]. Conventional mutations, such as point mutation, deletion, duplication, and frameshift, account for the remaining SCA subtypes.

Recently, SCA type 47 (SCA47) (MIM #617931) was found to be caused by mutations in the *PUM1* gene (MIM *607204), which encodes the Pumilio1 protein [3]. Pumilio1 belongs to a large family of RNA-binding proteins (RBPs) known as the Pumilio/FBF (PUF) family, characterized by the presence of an RNA-binding Pumilio homology domain that can bind to a highly conserved motif containing eight nucleotides [4]. Through the interaction with their target mRNA, PUF proteins regulate mRNA stability and repress translation [5]. *ATXN1*, the causal gene of SCA1 (MIM #164400), is negatively regulated by *PUM1*. Therefore, *PUM1* mutations with a loss-of-function effect may

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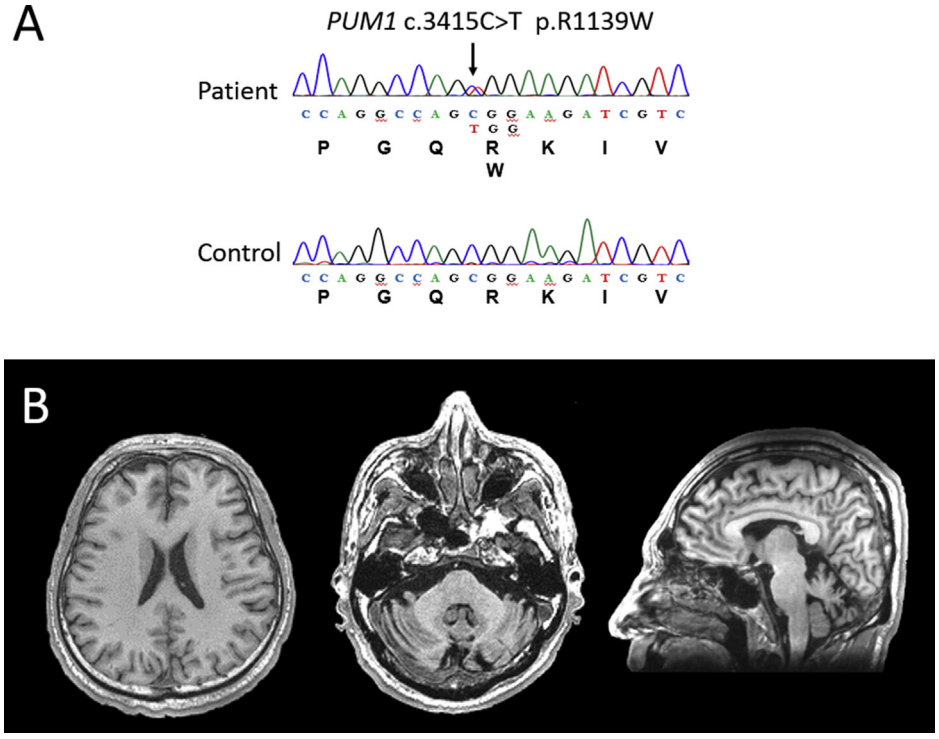

Fig. 1. (A) The pathogenic *PUM1* mutation, p.R1139W (c.3415C > T), identified in this study with the sense strand electropherogram shown on the top and a limited reading frame depicting the corresponding amino acid substitution shown below. (B) Features of T1-weighted brain magnetic resonance imaging (MRI) in the 46 year-old patient with spinocerebellar ataxia type 47.

lead to a higher *ATXN1* expression and subsequently result in cerebellar pathology [6].

SCA47 may present as two distinct clinical syndromes with different ages of disease onset. The adult-onset form of SCA47, originally named as *Pumilio1*-related cerebellar ataxia (PRCA), manifests a slowly progressive isolated cerebellar ataxia since age thirties to fifties years with an autosomal-dominant inheritance and incomplete penetrance. The missense *PUM1* mutation, p.T1035S, was found in a family with adult-onset subtype of SCA47 [3]. The early-onset SCA47, also known as *Pumilio1*-associated developmental disability, ataxia, and seizure (PADDAS), presents with developmental delay, intellectual disability, ataxia, and seizure. Heterozygous *PUM1* deletions and two de novo missense mutations (i.e. p.R1139W, p.R1147W) were found in patients with early-onset SCA47. Further functional study revealed that different degrees of Pumilio-1 deficiency result in corresponding clinical severities of SCA47.

Given that the clinical studies concerning *PUM1* mutations are still sparse and the contribution of mutations in *PUM1* to SCA or apparently sporadic cerebellar ataxia remains elusive, it would be intriguing to investigate *PUM1* mutations in patients with cerebellar ataxia of unknown cause. In the present study, we aimed at delineating the clinical features, frequency and spectrum of *PUM1* mutations in a Taiwanese cohort of 248 patients with cerebellar ataxia of unknown cause.

2. Methods

2.1. Patients

Two hundred forty-eight patients with cerebellar ataxia of unknown cause were enrolled from the Neurology service of Taipei Veterans General Hospital. The study participants included 108 unrelated patients with autosomal-dominantly inherited cerebellar ataxia, 45 index patients with autosomal-recessively inherited cerebellar ataxia, and 95 patients with apparently sporadic cerebellar ataxia. Patients were assigned to inherited cerebellar ataxia if he or she had one or more affected family members in two or more generations (autosomal dominant ataxia), in siblings (autosomal recessive ataxia), or with consanguineous parents (autosomal recessive ataxia). Patients who had a symptom onset between 20 and 40 years of age and negative family history, and denied any symptom of autonomic dysfunction were categorized into the apparently sporadic cerebellar ataxia group. Patients diagnosed with multiple system atrophy were excluded from this study. Individuals carrying a mutation responsible for SCA1, 2, 3, 6, 7, 8, 10, 12, 17, 19/22, 23, 26, 27, 28, 31, 35, 36, dentatorubral-pallidoluysian atrophy (DRPLA) and Friedreich's ataxia were excluded from the present study. All participants were of Han Chinese descent. All participants gave the written informed consent and the study was approved by the Institutional Review Board of Taipei Veterans General Hospital.

2.2. Mutation analysis

Genomic DNA was extracted from peripheral white blood cells. The coding exons and their flanking regions of *PUM1* were analyzed by PCR amplification and Sanger sequencing with the intronic primers using the Big Dye 3.1 dideoxy terminator method (Applied Biosystems, Foster City, CA, USA) on an ABI Prism 3700 Genetic Analyzer (Applied Biosystems). Amplicon sequences were compared with the reference *PUM1* coding sequence (NM_001020658.1). Since *PUM1*-associated ataxic syndrome should be extremely rare in the general population, the pathogenic variants were discriminated by their absence in the genome data of the 1517 health Taiwanese individuals from the Taiwan Biobank database (https://taiwanview.twbiobank.org.tw/index). We also queried the Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org) [7] for the variants. *In silico* predictions of the pathogenicity of the mutations were conducted using Mutation Taster (http://www.mutationtaster.org), polyphen-2 (http://genetics.bwh.harvard.edu/pph2) [8], SIFT (http://sift.jcvi.org), PROVEAN (http://provean.jcvi.org), and Combined Annotation Dependent Depletion (CADD) (http://cadd.gs. washington.edu).

3. Results

3.1. PUM1 variants in the cerebellar ataxia cohort

Mutational analyses of *PUM1* in the 248 patients with cerebellar ataxia revealed two heterozygous missense variants, namely

c.452A > G (p.K151R) and c.3415C > T (p.R1139W) (Fig. 1A). Each missense variant was identified in one single apparently sporadic case. Another eight synonymous variants were also found in the cerebellar ataxia cohort in a heterozygous form, including c.1395C > T (p.(P465 =)) and c.3201C > A (p. (I1067 =)) in two patients each, and c.132G > A (p. (S44 =), c.153T > C (p. (A51 =)), c.234C > T (p. (D78 =)), c.846A > G (p. (L282 =)), c.2337T > C (p. (N779 =)), and c.2379C > A (p. (A793 =) in one patient each.

3.2. Evaluation of the pathogenicity of the two PUM1 missense variants

Among the two missense variants, the pathogenicity of *PUM1* p.R1139W had been well demonstrated in the previous study [3]. The p.K151R variant was not found in the 1517 ethnically matched control genomes in the Taiwan Biobank database, but it was present with a low allele frequency of 0.0032% (8/251030) in the total population of gnomAD. Of note, the allele frequency is much higher in the East Asian subpopulation than other subpopulations of gnomAD (0.044%, 8/18388). The p.K151R is not located in the RNA-binding Pumilio homology domain, which is essential for the mRNA regulatory function of Pumilio1. *In silico* prediction of the pathogenicity of *PUM1* p.K151R showed variable results by different bioinformatics tools. Mutation Taster, polyphen-2, SIFT, and PROVEAN predicted the p.K151R variant to be disease-causing, probably damaging, tolerable, and neutral, respectively. The CADD v1.4 PHRED score was 28.6, suggesting that the p.K151R variant has a deleterious effect. Given the inconsistent *in silico* prediction results and a higher allele frequency of *PUM1* p.K151R in the East Asian population in GnomAD than that expected for SCA47, the pathogenicity of the p.K151R variant cannot be confirmed. Hence, the p.K151R was classified as a variant of uncertain significance (VUS) according to the American College of Medical Genetics (ACMG) guidelines [8]. The clinical information of the patient harboring *PUM1* p.K151R was described in the supplementary material.

3.3. Clinical information of the patient carrying PUM1 p.R1139W

Patient M903 is heterozygous for the *PUM1* p.R1139W mutation. He had a disease onset at age 35 years with slowly progressive gait unsteadiness and dysarthria. Neurological examination at age 46 years revealed mild saccadic pursuit, ataxic dysarthria, mild limb dysmetria and mild ataxic gait. He could walk independently but had difficulty in tandem walking. The score of scale for the assessment and rating of ataxia (SARA) score was 6.5 [9]. Brain MRI at age 46 years showed diffuse cerebellar atrophy with preserved cerebral and brainstem structures (Fig. 1B). Neurological examination at age 49 revealed similar findings with the same SARA score of 6.5. Mini-mental status examination (MMSE) demonstrated normal cognitive function with a score of 27 [10]. The patient reported no family history of cerebellar ataxia.

4. Discussion

To understand the contribution of *PUM1* mutations to cerebellar ataxia, we performed mutational analyses of *PUM1* in a Taiwanese cohort of 248 patients with cerebellar ataxia of unknown cause and found one pathogenic mutation, p.R1139W, in one patient with apparently sporadic cerebellar ataxia. We also identified another missense variant, *PUM1* p.K151R, but its pathogenicity is uncertain and its allele frequency in the East Asian population is greater than expected for SCA47. *PUM1* p.R1139W had been identified in a patient with early-onset SCA47 previously [3], and it was found in a patients with adult-onset cerebellar ataxia in the present study. The recurrence of *PUM1* p.R1139W in patients with cerebellar ataxia under different ethnic backgrounds provides compelling evidence for the pathogenic role of *PUM1* mutations in cerebellar ataxia. Furthermore, all participants in this study have been confirmed free of mutations responsible for 20 different inherited cerebellar diseases, including the most prevalent SCAs. Our findings suggest that *PUM1* mutations are not a frequent monogenic cause of cerebellar ataxia but still need to be considered as a possible cause of cerebellar ataxia of unknown cause.

The clinical manifestations and age of disease onset may vary largely among patients with a particular *PUM1* mutation. In our study, the man carrying a heterozygous *PUM1* p.R1139W mutation had a slowly progressive, relatively pure cerebellar ataxia since age 35 years. However, the same p.R1139W mutation also had been reported in a nine-year-old girl manifesting cerebellar ataxia, chorea and short stature before age 5 years. The phenotypic diversity also had been noted in patients harboring a heterozygous *PUM1* p.R1147W mutation. In the original SCA47 study, Gennarino et al. reported a nine-year-old girl carrying the p.R1147W mutation who was diagnosed with PADDAS, presenting with refractory epilepsy, progressive ataxia, lower limb hypotonia, global developmental delay, cortical visual impairment, stereotypic hand-clasping, scoliosis, facial dysmorphia, and osteoporosis since age 5 months [3]. Very recently, the same p.R1147W mutation was reported in another seventeen-year-old male patient with early onset developmental delay, epilepsy, microcephaly, and hair dysplasia [11]. His epilepsy developed at age 3 years and has been controlled well by medication. In addition to the variable clinical severity, *PUM1* mutations may have incomplete penetrance. In the previously reported SCA47 family with a heterozygous *PUM1* p.T1035S mutation, the four affected family members all have a slow progressive isolated cerebellar ataxia with a wide range of onset ages spanning from 30s to 50s years. Another 80-year-old lady within this family who also had the same *PUM1* mutation was asymptomatic, suggesting an incomplete penetrance.

The highly variable expressivity and/or incomplete penetrance of SCA47 in patients with a designated *PUM1* mutation indicate that there are some unknown genetic and/or environmental factors being capable of modifying the phenotypic presentation of SCA47. It would be very interesting to elucidate these factors, which may shed light on the molecular pathogenic mechanism of *PUM1*-associated cerebellar ataxia and provide hints to develop the specific therapeutic strategies.

In this study, we identified an additional case of adult-onset SCA47. The clinical manifestations of our patient are very similar to those reported previously. Adult-onset SCA47 manifests a very slowly progressive isolated cerebellar ataxia, which is consistent with the clinical classification of autosomal dominant cerebellar ataxia (ADCA) type III [12]. Among the ADCA III group, SCA6 is the most prevalent SCA subtype and other possible etiologies include SCA5, SCA10, SCA11, SCA14, SCA15, SCA22, SCA26, SCA30 and SCA31. Now, the differential list of ADCA III should be expanded to incorporate SCA47.

We might underdiagnose the SCA47 cases in our ataxia cohort because of limitation of the methodology. Firstly, we did not investigate deletions or duplications of total or a part of the *PUM1* gene in the cohort. Then, we could not recruit familial members of the patients with p.K151R variant or the eight *PUM1* synonymous variants for the co-segregation analyses. Since SCA47 mutations may have incomplete penetrance, we still cannot exclude its possible pathogenic role. On the other hand, some *PUM1* synonymous variants identified in this study might have potential influence on splicing (Supplementary Table). Further *in vitro* or *in vivo* functional analyses are warranted to elucidate the pathogenicity of these variants.

In conclusion, we identified a patient carrying a heterozygous *PUM1* p.R1139W mutation and suffering from an adult-onset slowly progressive cerebellar ataxia from a cohort of cerebellar ataxia of unknown cause. This study supports the pathogenic role of *PUM1* mutations and emphasizes the importance of considering *PUM1* mutations as a possible etiology of cerebellar ataxia.

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Declarations of interest

None.

Author's contribution

KLL: Study concept and design, acquisition of data, analysis and interpretation, manuscript writing. YCLiao: Study concept and design, patient recruitment, study supervision. PCT: Acquisition of data, analysis and interpretation, technical support. CTH: Patient recruitment, study supervision. BWS: Study concept and design, patient recruitment, study supervision, critical revision of the manuscript for important intellectual content. YCLee: Study concept and design, patient recruitment, study supervision, critical revision of the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.08.004.

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