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RESEARCH ARTICLE

A novel *de novo* mutation in the *PURA* gene associated with a new clinical finding: large brainstem

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Abstract. We report the case of a Caucasian Spanish boy, who showed profound neonatal hypotonia, feeding difficulties, apnea, severe developmental delay, epilepsy, bilateral convergent strabismus, poor verbal language development and a large brainstem. Whole-exome sequence uncovered a novel *de novo* mutation in the purine-rich element binding protein A gene (*PURA*; NM_005859.4:c.72del:p.(Gly25AlafsTer53)) that encodes the transcriptional activator protein Pur-alpha (PURA). Mutations in this gene have been identified in patients with PURA syndrome, a rare disorder characterized by an early hypotonia, developmental delay, severe intellectual disability with or without epilepsy, and disability in expressive language development. Although, up to 75 cases have been identified worldwide, to the best of our knowledge, this is the first patient described with a brainstem larger than normal. In conclusion, our data expand both genetic and phenotypic spectrum associated with *PURA* gene mutations.

Keywords. *PURA* gene; whole-exome sequence; brainstem; hypotonia; developmental delay; epilepsy.

Introduction

Purine-rich element binding protein A gene (*PURA*; MIM: 600473) is a single exon gene that encodes the transcriptional activator protein Pur-alpha (PURA; Uniprot: Q00577). PURA is a ubiquitously expressed member of the Pur family of nucleic acid binding proteins that is involved in neuronal proliferation, dendrite maturation, and the transport of mRNA to translation sites during neuronal development translation (Tanaka *et al.* 2015). Heterozygous mutations in *PURA* gene are responsible for PURA syndrome which is characterized by moderate to severe neurodevelopmental delay, motor delay, hypotonia, language delay, feeding difficulties, apneas, epileptic seizures, abnormal nonepileptic movements, visual problems, and less common, congenital heart defects, urogenital malformations, skeletal abnormalities, and endocrine disorders. To date, 60 different mutations in the *PURA* gene have been characterized in 75 individuals with PURA syndrome (Hunt *et al.* 2014; Lalani *et al.* 2014; Tanaka *et al.* 2015; Okamoto *et al.* 2017; Rezkalla *et al.* 2017; Lee *et al.* 2018; Mayorga *et al.* 2018; Reijnders *et al.* 2018; Qiao *et al.* 2019).

Here, we described a Spanish patient with a novel *de novo* mutation in *PURA* gene that presents a radiological finding undescribed before, expanding the genotype and phenotype associated to the PURA syndrome.

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Methods

Clinical report

The Ethic Committee of the Instituto de Investigación Hospital 12 de Octubre (i+12) approved the study of the patient, and written informed consent was obtained from the patient's parents.

A 17-years-old male was referred to our clinic at the age of 6 with severe developmental delay, epilepsy and bilateral convergent strabismus. He was the second son of nonconsanguineous healthy parents and his older brother was also healthy. The mother had a history of metrorrhagia in the first trimester of pregnancy. The patient was born after 42 weeks of gestation by vaginal delivery and induced labour in the context of post-term pregnancy and loss of foetal well-being. The Apgar score was not available, but neonatal resuscitation maneuvers were performed. Birth weight was 4.2 kg (+1.6 SD), birth length 54 cm (+1.8 SD), but cranial perimeter was not registered. He presented with profound neonatal hypotonia and feeding difficulties requiring nasogastric tube feeding during the first week. At two months of age, he was admitted with episodes of apnea during a respiratory syncytial virus infection, requiring the use of continuous positive airway pressure. In the following months a psychomotor delay was observed, he held his head up at 9 months, acquired the sitting position at the age of 3 years and independent ambulation at the age of 5 years. He also had intellectual disability with poor verbal language development that was limited to rare vocalizations. At the age of 6, he started with refractory generalized epilepsy to the treatment. In the follow-up, the child presented a slow neurological regression. At the age of 14 years, he was nonverbal, nonambulatory and his ability to contact was very poor, during which his weight was 60 kg (−0.3 SD), height 155 cm (−1.9 SD), and cranial circumference 56 cm (0.1 SD). Physical examination revealed myopathic face with an open mouth, excessive drooling, generalized hypertonia with bilateral knees flexion position, exaggerated startle response, pyramidal signs, a subluxation of right hip and severe thoracolumbar scoliosis. Until now, he was under treatment with lamotrigine, vigabatrin, eslicarbazepine acetate and coenzyme Q10. Blood gases, glucose, transaminases, creatinine, homocysteine and CRF study were normal. The metabolic screening was also normal. Magnetic resonance imaging (MRI) was performed at the age of 15 (see below). Cardiological studies showed no relevant findings. The ophthalmologic examination showed only bilateral convergent strabismus with normal fundoscopy. Muscle biopsy analysis showed no histological or histochemical abnormalities, and the activities of mitochondrial respiratory chain complexes were normal. A deficiency of coenzyme Q10 in fibroblasts and muscle was observed. The aCGH (comparative genomic hybridization array) analysis and the whole mtDNA sequencing did not show any pathological variant.

Genetic analysis

Whole-exome sequencing (WES) was performed (BGI, Hong Kong) on genomic DNA obtained from the patient following a standard protocol. Briefly, the amplified DNA fragments were hybridized to the Agilent SureSelect Human All Exon V4 (51 Mb), the captured library was sequenced on a HiSeq 2000 platform, and the reads were aligned against the human reference genome (GRCh37 at UCSC) to obtain candidate variants. Nuclear variants and indels were prioritized according to the following criteria: (i) variants consistent with a recessive model of pathogenesis: (a) variants that were rare in healthy individuals (allele frequency below 0.01) or new (not described within public databases); (b) variants predicted to modify protein function (nonsense, splice site, coding indel, or missense variants); (c) variants consistent with a recessive model of pathogenesis: homozygous variants or two heterozygous variants present in the same gene. (ii) Variants consistent with a dominant model of pathogenesis: (a) variants that were very rare in healthy individuals (allele frequency below 10^{-5}) or new; (b) variants predicted to modify protein function; (c) variants consistent with a dominant model of pathogenesis: heterozygous variants.

Additional indications to prioritize the candidate genes were obtained by using predictive software scoring the likelihood for pathogenicity SIFT, Polyphen-2, MutPred and Variant Taster. Sanger sequencing of the patient, his brother and his parents was performed for candidate gene variant.

Results

Brain MRI findings

The MRI performed at the age of 15, showed no lesions at the brain, no malformations, but the brainstem seems to be larger than normal. We have measured the diameters of mesencephalon, pons and medulla oblongata and compared them with published measures what confirmed our supposition (figure 1a; table 1) (Raininko *et al.* 1994; Elhussein *et al.* 2017; Yoshida *et al.* 2017; Garbade *et al.* 2018). Paradoxically, supratentorial findings showed large ventricles, incremented extra-axial space at the opercula (opercular dysplasia), width diploe and big pneumatized frontal sinuses. All these supratentorial findings are related with certain degree of supratentorial atrophy (figure 1, b–c).

Sequencing results

The aforementioned analytic pipeline for nuclear variants and indels analysis was used to prioritize variants in genes that were rare and were predicted to be deleterious. We found a heterozygous single nucleotide deletion (NM_005859.4:c.72del:p.(Gly25AlafsTer53) in exon 1 as

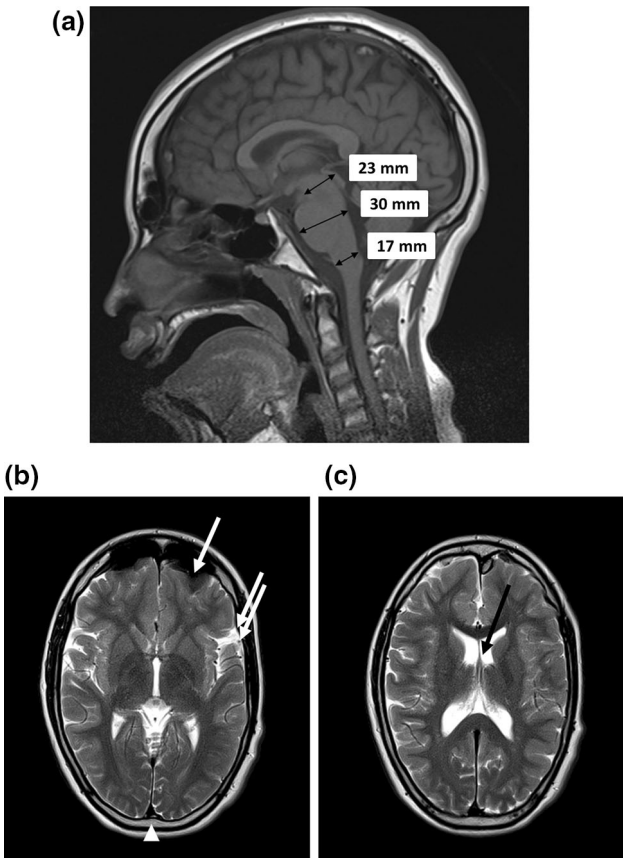

Figure 1. MRI brain scans of patient. (a) Mid sagittal T1-weighted image shows measures of the brain stem larger than normal. (b) Axial T2-weighted image at the level of opercula showed big pneumatized frontal sinus (white arrow), width diploe (arrowhead), and opercular dysplasia (double white arrow). (c) Axial T2-weighted image at the level of lateral ventricles showed large ventricles (black arrow).

numbered in NG_041813.1) in the purine-rich element binding protein A gene (*PURA* gene; MIM: 600473) that encodes for the transcriptional activator protein Pur-alpha (PURA; Uniprot: Q00577). Sanger sequencing of patient, his brother and his parents was performed, and the NC_000005.9:g.139493835delC (chr5, hg19) mutation was found to be *de novo* (figure 2, a–b).

Discussion

Here, we describe a Spanish child with profound neonatal hypotonia, feeding difficulties, apnea, severe developmental delay, epilepsy, bilateral convergent strabismus with an unexpected new finding, namely a large brainstem. Using WES, we were able to identify a heterozygous mutation in the nuclear-encoded gene *PURA*. This frameshift mutation (p.(Gly25AlafsTer53)) was found to occur as *de novo* origin (figure 2a), neither previously reported nor described in any genomic database (1000G, EVS, ExAC Browser, gnomAD browser and ClinVar Database). The predicted outcome of this frameshift is a truncated dysfunctional protein with 76 aa, where the first 25 are the same of the PURA, but the rest are different (figure 2c).

PURA is an ubiquitously expressed protein (including the brain, muscle, heart and blood) that binds specific sequences of ssDNA, dsRNA, and ssRNA (Tanaka *et al.* 2015). It is a 322 aa protein that contains three highly conserved PUR repeats (I-III) (figure 2c) and regulates a variety of cellular processes including DNA replication, gene transcription, RNA transport, and mRNA translation (Lalani *et al.* 2014). Therefore, PURA is a transcription factor expressed during early brain development (Lee *et al.* 2018), and mutations in *PURA* gene have been documented to cause neurodevelopmental disorders. Thus, 'mental retardation, autosomal dominant 31 (MRD31; MIM:616158)' or PURA-related neurodevelopmental disorders include PURA syndrome (~90% of affected individuals), caused by a heterozygous mutation in *PURA* gene, and 5q31.3 deletion syndrome (~10% of affected individuals), caused by a genomic deletion encompassing all or part of *PURA* gene (Reijnders *et al.* 2018). PURA syndrome is characterized by moderate to severe neurodevelopmental delay, motor delay (nonambulatory or severely delayed), hypotonia, language delay (nonverbal), feeding difficulties, neonatal respiratory

Table 1. Brainstem measurements.

		Diameter (sagittal)		
	Patient	Mesencephalon 23.0 mm	Pons 30.0 mm	Medulla oblongata 17.0 mm
Garbade *et al.* (2018)	Controls	18.6 ± 1.2	22.3 ± 1.2	12.5 ± 0.7
	Patient	+3.7 SD	+6.4 SD	+6.4 SD
Elhussein *et al.* (2017)	Controls	16.0 ± 1.5	22.0 ± 1.6	13.4 ± 1.1
	Patient	+4.7 SD	+5.0 SD	+3.3 SD
Yoshida *et al.* (2017)	Controls	16.3 ± 1.3	22.5 ± 1.7	12.3 ± 1.5
	Patient	+5.1 SD	+4.4 SD	+3.1 SD
Raininko *et al.* (1994)	Controls	17.0 ± 1.0	22.5 ± 1.4	13.4 ± 1.0
	Patient	+6.0 SD	+5.4 SD	+3.6 SD

Controls, mean ± SD from the indicated references; patient, (patient's diameter – mean)/SD.

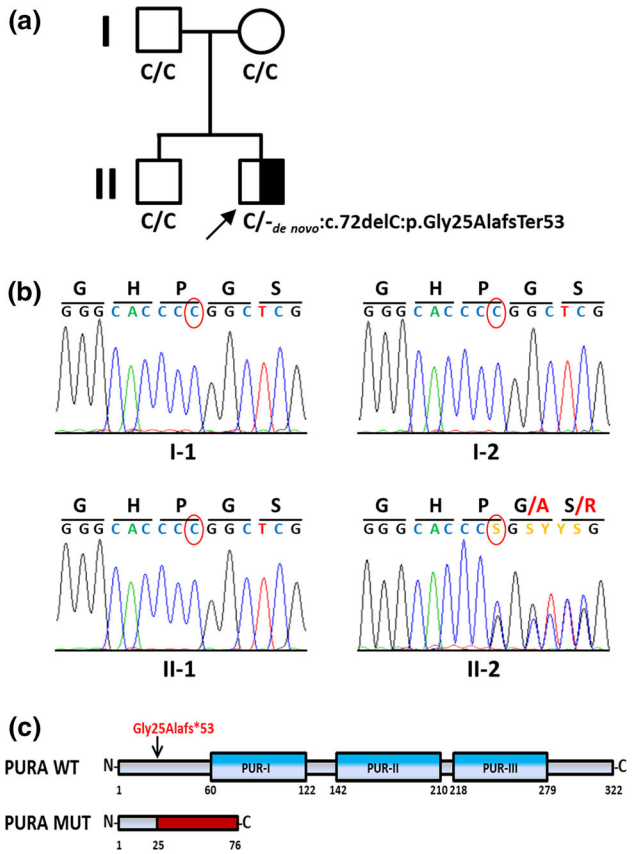

Figure 2. Genetics findings of the patient. (a) Family pedigree showing the genotype of the *de novo* c.72delC (p.Gly25AlafsTer53) mutation in *PURA* gene (NM_005859.4). (b) Electropherograms showing Sanger sequence validation of the *PURA* c.72delC (p.Gly25AlafsTer53) mutation. (c) Schematic representation of human transcription activator protein Pur-alpha (PURA) showing the position of p.Gly25AlafsTer53 (red) mutation, and the three PUR motifs showing (blue) (PURA WT). The mutation produces a shorter peptide of 76 aa, where the first 25 are the same of the PURA, but the rest are different (red) (PURA MUT).

difficulty and epilepsy. Other less common findings are congenital heart defects, urogenital malformations, skeletal abnormalities, craniofacial dysmorphism and endocrine disorders. The most frequent MRI finding in these patients is delayed myelination, while the excessive extra-axial fluid spaces and volume loss of the corpus callosum are less frequent. Currently, 75 patients with 60 different mutations in *PURA* gene have been characterized so far, and all of them have been diagnosed with PURA syndrome (Hunt *et al.* 2014; Lalani *et al.* 2014; Tanaka *et al.* 2015; Okamoto *et al.* 2017; Rezkalla *et al.* 2017; Lee *et al.* 2018; Mayorga *et al.* 2018; Reijnders *et al.* 2018; Qiao *et al.* 2019). However, there are ∼295 diagnosed cases of PURA syndrome worldwide (www.purasyndrome.org).

Our patient has a *de novo* mutation (p.Gly25AlafsTer53) in *PURA* gene that has not been previously described. In addition, an MRI brain scan of the patient, showed a brainstem larger than normal, including the diameters of mesencephalon, pons and medulla oblongata greater (figure 1a; table 1) (Raininko *et al.* 1994; Elhussein *et al.* 2017; Yoshida *et al.* 2017; Garbade *et al.* 2018). This abnormality in the brainstem diameter, to the best of our knowledge, has not been previously described in any other patient with a *PURA* gene mutation.

Two knock-out of *PURA* gene in mice have been described (Khalili *et al.* 2003; Hokkanen *et al.* 2012), and although the phenotype is similar (severe tremor), the consequences at cellular level in brain are different. Thus, one of them reported a severe reduction in the proliferation of neural precursor cells in postnatal brain development (Khalili *et al.* 2003), whereas the other observed a significant enhancement of proliferation (Hokkanen *et al.* 2012). Accordingly, an alteration in proliferation of neural precursor cells (an increase or a decrease) in postnatal brain due to a haploinsufficiency of *PURA* gene, is the responsible for the neurological problems manifest by the patients. In our patient, the reduction in the proliferation would be responsible for supratentorial atrophy, and an enhancement of proliferation in brainstem would be responsible for its hypertrophy and increase size. As this observation is from a single patient, it is not possible to be certain this change is a feature of PURA syndrome patients, and further studies are required to clarify this genotype–phenotype association.

In conclusion, our findings expand both the genetic and phenotypic spectrum of *PURA*-associated diseases.

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