

# Expanding The Clinical Phenotype and Genetic Spectrum of PURA-Related Neurodevelopmental Disorders

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## Research

**Keywords:** PURA neurodevelopmental disorders, 5q31.3 microdeletion syndrome, PURA syndrome, PURA, Global developmental delay and intellectual disability (GDD/ID)

**Posted Date:** February 8th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-163113/v1>

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## Abstract

**Background:** PURA-related neurodevelopmental disorders (PURA-NDDs) include 5q31.3 deletion syndrome and PURA syndrome. PURA-NDDs are characterized by neonatal hypotonia, moderate to severe global developmental delay/intellectual disability (GDD/ID), facial dysmorphism, epileptic seizures, nonepileptic movement disorders, and ophthalmological problems. PURA-NDDs have recently been identified and underestimated in neurodevelopmental cohorts, but their diagnosis is still challenging. We retrospectively reviewed the clinical characteristics, genetic spectrum, and diagnostic journey of patients with PURA-NDDs.

**Results:** We report 2 patients with 5q31.3 microdeletion and 5 with *PURA* pathogenic variants. They demonstrated hypotonia (7/7, 100%), feeding difficulties (4/5, 80%), and respiratory problems (4/7, 57%) in the neonatal period. All of them had severe GDD/ID and could not achieve independent waking and verbal responses. Distinctive facial features of open-tented upper vermillion, long philtrum, and anteverted nares and poor visual fixation and tracking with or without nystagmus were most commonly found (5/7, 71.4%). There were no significant differences in clinical phenotypes between 5q31.3 microdeletion syndrome and PURA syndrome. PURA-NDDs need to be considered as a differential diagnosis in individuals who show severe hypotonia, including feeding difficulties since birth and severe developmental retardation with distinctive facial and ophthalmological features.

**Conclusions:** Our data expands the phenotypic and genetic spectrum of PURA-NDD. Next-generation sequencing methods based on the detailed phenotypic evaluation would shorten the diagnostic delay and would help this rare disorder become a recognizable cause of neurodevelopmental delay.

## Introduction

PURA-related neurodevelopmental disorders (PURA-NDDs) include 5q31.3 deletion syndrome and PURA syndrome which is caused by a *PURA* pathogenic variant [1]. The 5q31.3 deletion syndrome was initially described as neonatal hypotonia, severe global developmental delay/intellectual disability (GDD/ID), distinctive facial features, epileptic seizures, and nonepileptic movement disorders [2-4]. Within this region, purine-rich element binding protein A (*PURA*) has been suggested as a critical causative gene [5, 6]. The *PURA* gene (NM\_005859) encodes a highly conserved Pur-alpha protein that plays a critical role in neuronal development and differentiation [7, 8].

There is no doubt that chromosomal microarray (CMA) as a first-tier diagnostic test enhances the diagnostic yield for patients with GDD/ID [9, 10]. Whole-exome sequencing (WES) also provided new opportunities to diagnose sporadic de novo variants in neurodevelopmental disorders [11, 12]. PURA-NDDs have recently been identified and may be underestimated, accounting for less than 1% of the cause of neurodevelopmental delay [13-15]. There are universal clinical features of PURA-NDD; however, associated features expanding the phenotypes of PURA-NDDs are increasingly reported [16, 17]. Furthermore, most reported PURA-NDD patients are in their infancy or childhood, and the natural clinical course of PURA-NDDs is rarely reported.

We report 2 individuals with the 5q31.3 deletion and 5 individuals with de novo pathogenic variants in *PURA*. We described their clinical features, including the detailed neurodevelopmental history and genetic spectrum. We retrospectively reviewed the diagnostic journey that individuals went through for etiologic and differential diagnoses.

## Methods

### Patients and study approval

We identified 7 patients with PURA-NDD from our cohort who were referred to the pediatric neurology clinic for unexplained GDD/ID. Six of them participated in the Korean Undiagnosed Disease Program and were diagnosed with PURA-NDDs [18]. All medical records, including birth history, detailed developmental history, and brain magnetic resonance imaging (MRI), were reviewed retrospectively. This study followed the Declaration of Helsinki's principles and was approved by the institutional review board of Seoul National University Hospital (IRB No. 1912-136-1091).

### Molecular analysis

Blood samples were obtained from enrolled patients and their parents who provided informed consent. Genomic DNA was extracted from peripheral blood samples using a QIAamp DNA Blood Midi Kit (Qiagen, Valencia, CA, USA). CMA testing was performed with either the Agilent Human Genome oligonucleotide comparative genomic hybridization (CGH) microarray 8x60K (Agilent Technologies, Santa Clara, CA, USA) or the Cytoscan Dx Assay (Affymetrix, Thermo Fisher Scientific) according to the manufacturer's instructions. Array data were analyzed using Genomic Workbench software (version 7.0.4.0, Agilent Technologies). Whole-exome sequencing (WES), including exome capture and sequencing, was performed at the Theragen Etx Bio Institute (Suwon, Korea) following the standard protocol. Sequenced reads were aligned to the hg19 reference genome with Burrows-Wheeler Aligner (v.0.7.15) and were processed with Picard software (v2.8.0), SAMtools (v.1.8) and Genome Analysis Toolkit (GATK, v.4.1.4). Variants were annotated using ANNOVAR. The pathogenicity of variants was evaluated according to the American College of Medical Genetics (ACMG) standard guidelines and annotated by InterVar [19, 20]. All of the *PURA* variants identified by WES were confirmed by Sanger sequencing. Segregation tests were performed to confirm de novo occurrence of the pathogenic *PURA* variants.

## Results

### Clinical features with developmental outcome

There were 2 patients with 5q31.3 deletion syndrome and 5 patients with *PURA* variants. Detailed clinical features and genetic information are summarized in Table 1. Neonatal problems were evident in all individuals immediately after birth. All of them had profound neonatal hypotonia (7/7, 100%). Feeding difficulties requiring tube feeding (4/5, 80%) and apnea and/or respiratory distress (3/7, 43%) were associated in the neonatal period. All patients had severe

gross motor delay, and they never attained independent walking (7/7, 100%) at the last follow-up (age range 3-18 years). The maximal motor function in our patients was standing with support at age 3 years or older. All individuals had severe language impairment in that they could not speak any single words (7/7, 100%). Non-epileptic movements, including exaggerated startling responses and jerky movements, were associated in 43% (3/7) of individuals. Two patients (2/7, 28.5%) had epileptic seizures that were well controlled with conventional anti-seizure medications. On reviewing brain MRIs, the most frequently observed feature was widening of the subarachnoid spaces, suggesting decreased cerebral volume (6/7, 86%). Delayed myelination was also observed on MRI in 29% (3/7) of patients. Five of 7 patients (71.4%) presented distinctive facial features including open-tented upper vermilion, long philtrum, and depressed nasal bridge with anteverted nares. They also had ophthalmological problems such as poor visual fixation and tracking with or without nystagmus. On polysomnography, patient No.1 had central apnea that required bilevel positive airway pressure. Patient No.2 was the oldest patient in our cohort and was found to have primary amenorrhea. Her pubertal development at 17 years of age was Stage 4 (breasts and pubic hair) in Tanner staging; however, she did not have menarche.

### Diagnostic journey

We reviewed all patients comprehensively and shared their diagnostic journey and their developmental outcome (Table 1). Prior to the identification of PURA-NDD, patients underwent evaluation related to Prader-Willi syndrome (4/7, 57%), metabolic screening (4/7, 57%), or spinal muscular atrophy (2/7, 29%) in their neonatal or infancy periods. Five of seven patients (71.4%) underwent muscle biopsies, electromyography, or nerve conduction studies due to severe hypotonia with feeding difficulty and facial weakness. All patients underwent initial diagnostic evaluation in their neonatal period and were referred to our center before age of 2 years. However, it took an average of 5 years (range 1-16 years) to obtain the genetic diagnosis of PURA-NDDs from the time they were referred to our center. For example, we could not diagnose patient No. 2 in the neonatal period, which eventually caused the patient to be lost to follow-up for 15 years. Prior to the identification of PURA-NDD, she was considered to have cerebral palsy and continued physical therapy. New-onset epileptic seizures at age 17 brought her to our clinic. Finally, we diagnosed her with 5q31.3 microdeletion syndrome based on a chromosomal microarray.

### Genetic spectrum

Patient No.1 had a small interstitial deletion of 0.45 Mb in region 5q31.3 (139,037,798-139,495,071), and patient No. 2 had an interstitial deletion of approximately 2.5 Mb at 5q31.2-31.3 (139,037,798-139,495,071). Parental testing was not available in these two patients. However, the deletions overlapped with previously reported regions and regions including the *PURA* (Figure 1A) We found four missense and two nonsense variants from five patients, which were all de novo (Figure 1B). Patient No. 5 interestingly had two pathogenic *PURA* variants (c.228C>G, c.331C>T) that were located in *cis*.

## Discussion

PURA-related NDD has been recently recognized as a cause of ID with advances in genetic technologies. To date, 11 patients with 5q31.3 deletion syndrome and 85 patients with PURA syndrome, including our patients, have been reported. The universal clinical features of PURA-NDDs are moderate to severe ID with verbal language delay and profound neonatal hypotonia [15]. We confirmed that all of our patients with PURA-NDDs displayed hypotonia in the neonatal period. Their developmental milestones regarding gross motor and language were severely retarded beyond the neonatal period. All patients in our cohort were not able to attain independent gait even with appropriate physical therapy, similar to most previously reported patients (1). Some earlier studies described patients with *PURA* variants who were able to achieve their first steps from 28 months to 7 years and even maintained independent gait at age 20 [13, 16]. Language delays were evident in all patients, as they were nonverbal. Cinquina et al. summarized other associated clinical features, including skeletal, cardiac, urogenital, and dermatological abnormalities, in PURA syndrome [15]. They mentioned that distinctive facial traits were reported in 84.5% of patients with PURA syndrome. As demonstrated by computational analysis, dysmorphic facial features include myopathic faces, full cheeks, high anterior hairlines, shorter palpebral fissures, and prominent philtrums [13]. Concordant with earlier studies, 71.4% of our patients had distinctive facial features with typically open-tented upper vermilion, long philtrum, and anteverted nares. Ophthalmological problems such as nystagmus, poor visual fixation and tracking were also commonly associated in our patients. The prevalence was higher than that in the pre-existing literature, in which strabismus was an accompanying symptom in approximately 30-40% of individuals with PURA-NDDs [6, 13, 14]. Exaggerated startling responses were associated in 43% of patients, which was similar to previous reports [15]. Endocrine abnormalities such as aberrant sex and thyroid hormone levels have also been reported in PURA-NDD [13, 21]. Bonaglia et al. presented a 26-year-old female who had a 5q31.2q31.3 microdeletion and had pubertal delay and primary amenorrhea [22]. Additionally, Reijnders et al. reported two patients (Patient No.1 and Patient No.23) who had hypogonadotropic hypogonadism that required medical treatment [1]. Very recently, Boczek et al. published a case study of a 20-year-old male with short stature and delayed puberty who had hypogonadotropic hypogonadism requiring supplementation [16]. At present, the majority of reported PURA-NDD patients are in their childhood, and little is known about endocrine problems beyond the age of puberty in PURA-NDDs. Endocrine problems related to sex hormones could be one of the clinical features in patients with PURA-NDDs and would be worth monitoring in patient care and exploring in future studies.

PURA-NDD was first described and identified by microarray-based CGH [2]. As WES has been gradually expanded in the clinical setting, a number of PURA syndromes caused by de novo pathogenic variants have been increasingly reported [5, 6, 14]. PURA-NDDs do have core clinical phenotypes. Reijnders et al. depicted a striking example of diagnosing PURA syndrome in a neonate through targeted Sanger sequencing based on clinical phenotype [13]. However, there is still a broad spectrum of clinical features and variability in clinical severity within PURA-NDD. These variabilities challenge the diagnostic process even for specialized clinicians. When we retrospectively reviewed the diagnostic process, more than half of patients underwent evaluations under the impression of central or neuromuscular hypotonia such as Prader-Willi syndrome, inherited myopathies and metabolic disorders in their neonatal period due to hypotonia, feeding difficulty, and respiratory problems. In their infancy, more than 70% of patients had muscle biopsies and electrophysiological studies to rule out neuromuscular disorders. Reviewing other works, clinicians frequently performed single-gene testing of Prader-Willi syndrome, Fragile X syndrome (*FMR1*), metabolic testing and occasional mitochondrial studies [1, 16]. Similar to our study, muscle biopsies and myotonic dystrophy testing were also frequently performed. Due to respiratory problems or irregular breathing patterns, a central hypoventilation panel or *PHOX2* sequencing was applied in a few patients. We

suggest that PURA-NDDs need to be considered as a differential diagnosis in patients who have severe neonatal hypotonia with feeding and respiratory difficulty, followed by profound global developmental delay, particularly in verbal expression.

We did not observe differences in the severity of clinical features between individuals with 5q31.3 microdeletions and single-nucleotide pathogenic variants in *PURA*. Our study supports that *PURA* is a primary causative gene for the core neurodevelopmental features of 5q31.3 microdeletion syndrome [5]. Similar to previous studies, we could not find correlations between the types or locations of variants and clinical severity and variability [13, 23]. Patient No.5 had two pathogenic variants, and the phenotypic severity was not different from the others. This mechanism is suggested by a dominant-negative effect for structural variants and functional haploinsufficiency for truncating mutations. This might be explained by the involvement of other genetic or biological mechanisms.

## Conclusions

*PURA*-NDDs have universal clinical features of neonatal hypotonia and severe GDD/ID with severely impaired verbal expression. Based on the detailed clinical phenotypes, *PURA*-NDD is gradually being illustrated in neurodevelopmental disorders. Thus, we should be able to obtain increasing amounts of information about the natural course of disease, which would lead to earlier diagnosis and better patient care.

## Declarations

### Ethics approval and consent to participate

This study protocol was in accordance with the tenets of Declaration of Helsinki and was approved by the Institutional Review Board of Seoul National University.

### Consent for publication

Not applicable

### Availability of data and materials

All data generated or analyzed for the study are available from the corresponding author upon reasonable request.

### Competing interests

The authors have no conflict of interest of financial relationship to disclose.

### Funding

This study was supported by intramural grants from the Korea National Institute of Health (Grant No.2019-NG-055-01).

### Author's contributions

CSA and HSL prepared the first draft of the manuscript and edited the manuscript drafts. TJP and YJK contributed to the data acquisition. SP and SYK managed all the process of CMA, WES and Sanger sequencing. SP and HSL analyzed the WES data. BCL and KJK supervised the study and critically reviewed the manuscript. JHC conceived of the study and reviewed edited the manuscript drafts until the final draft was produced. All authors read and approved the final manuscript.

### Acknowledgements

We appreciate the contributions to DNA preparation by the pediatric neurology laboratory at Seoul National University Children's Hospital.

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## Table

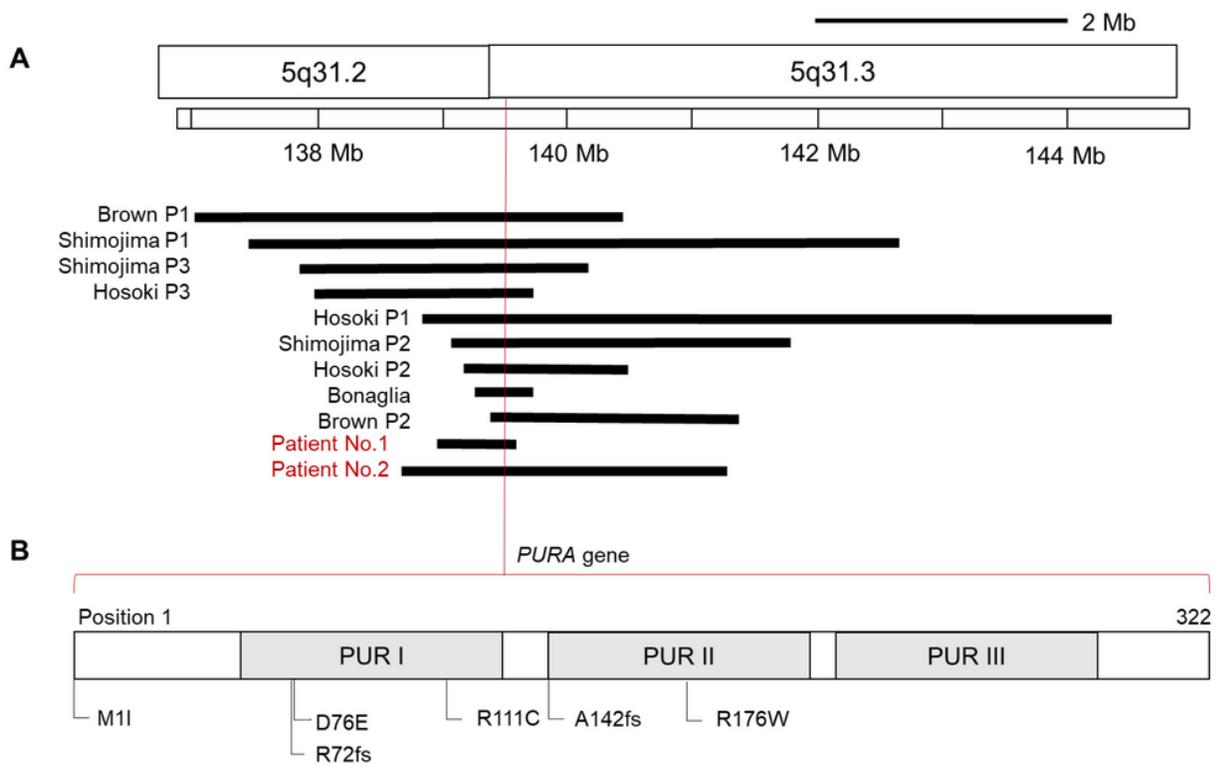
**Table 1.** Clinical features and genetic spectrum of patients with PURA neurodevelopmental disorders

Patient No.	1	2	3	4	5	6	7
Deletion	5q31.3	5q31.2q31.3					
Size (Mb)	0.45	2.5					
Nucleotide alteration			c.3G>T	c.211_214dupAAGC	c.228C>G, c.331C>T	c.407_420dupAGGACGAGCCGCGC	c.526C>T
Amino acid substitution			p.M1I	p.Arg72fs	p.Asp76Glu p.Arg111Cys	p.Ala142fs	p.Arg176Glu
Origin	N/A	N/A	De novo	De novo	De novo	De novo	De novo
Gender	Female	Female	Female	Male	Male	Male	Male
Age at last follow-up (years)	9	18	4	5	4	9	3
Neonatal features							
Hypotonia	+	+	+	+	+	+	+
Feeding difficulties	+	+	+	+	N/A	N/A	-
Respiratory problems	Apnea	Respiratory distress, apnea	Intermittent perioral cyanosis	-	-	+ Respiratory distress	-
Development							
Motor developmental delay	Severe	Severe	Severe, roll over and sit with support at 21 mo	Severe, stand with holding at 28 mo	Severe, stand with holding at 43 mo	Severe	Severe, stand with holding at 35 mo
Independent ambulation (at last FU age)	-	-	-	-	-	-	-
Speech (at last FU age)	Nonverbal	Nonverbal	Nonverbal	Nonverbal	Nonverbal	Nonverbal	Nonverbal
Neurological features							
Nonepileptic movement	+, Startling and jerking	+, Hyperekplexia	-	-	-	-	+, Myoclonic jerk
Epilepsy, age of onset	+, 9 mo	+, 17 yr	-	-	-	-	-
EEG	Slow BGA	Slow BGA	N/A	Normal	N/A	Normal	Normal
Brain MRI	Decreased volume	Myelination delay	Minimal widening of subarachnoid space	Widening of subarachnoid space, myelination delay	Minimal widening of subarachnoid space	Widening of subarachnoid space, myelination delay	Widening of subarachnoid space
Others							
Ophthalmologic features	+, Nystagmus and eye rolling	+, Strabismus, nystagmus and eye rolling	+, Nystagmus and eye rolling	+, Nystagmus	N/A	+, Strabismus	N/A
Facial features	+, Open-tented upper vermillion	+, Open-tented upper vermillion, long philtrum, bilateral ptosis, anteverted nares	+, Open-tented upper vermillion, apathic face	+, Open-tented upper vermillion, long philtrum, anteverted nares	-	+, Long philtrum, anteverted nares	-
	Central apnea	Primary amenorrhea					
Diagnostic journey							
Duration between 1 <sup>st</sup> clinic visit to	5 years	16 years	1 year	3 years	1 year	6 years	2 years

final genetic diagnosis							
Tests performed before diagnosis	SMA, PWS, metabolic testing, muscle biopsy, WES	Karyotyping	Karyotyping, PWS, myotonic dystrophy, SMA, muscle biopsy, metabolic testing, EMG, NCS	Karyotyping, metabolic testing, PWS	Karyotyping, CMA, EMG, NCS	Karyotyping, CMA, PWS	Karyotyping, metabolic testing, muscle biopsy, EMG, NCS
Test used for diagnosis	CMA	CMA	WES (trio)	WES (proband)	WES (trio)	WES (proband)	WES (trio)

Abbreviation: N/A – not available; mo – months; EMG – electromyography; EEG – electroencephalography; BGA – background activity; MRI – magnetic resonance image; PWS – Prader-Willi syndrome; SMA – spinal muscular atrophy; EMG – electromyography; NCS – nerve conduction study, CMA – chromosome microarray, WES – whole-exome sequencing

## Figures



**Figure 1**  
 (A) Overview of previously reported cases with 5q31.3 deletions and our patients' deletions (Patients No.1 and 2). (B) PURA structure and protein domains of Pur I-III. All pathogenic PURA variants identified from our study are shown in the protein diagram.