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PURA- Related Developmental and Epileptic Encephalopathy: Phenotypic and Genotypic Spectrum

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PURA-Related Developmental and Epileptic Encephalopathy

Phenotypic and Genotypic Spectrum

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Glossary

AA = amino acid; **ASM** = antiseizure medication; **DEE** = developmental and epileptic encephalopathy; **ESES** = electrical status epilepticus during sleep; **GTC** = generalized tonic-clonic; **ID** = intellectual disability; **KO** = knockout; **LEV** = levetiracetam; **PTV** = protein-truncating variant; **PURA** = purine-rich element-binding protein A; **SUDEP** = sudden unexpected death in epilepsy; **TPM** = topiramate; **VPA** = valproate.

Abstract

Background and Objectives

Purine-rich element-binding protein A (*PURA*) gene encodes Pur-α, a conserved protein essential for normal postnatal brain development. Recently, a *PURA* syndrome characterized by intellectual disability, hypotonia, epilepsy, and dysmorphic features was suggested. The aim of this study was to define and expand the phenotypic spectrum of *PURA* syndrome by collecting data, including EEG, from a large cohort of affected patients.

Methods

Data on unpublished and published cases were collected through the *PURA* Syndrome Foundation and the literature. Data on clinical, genetic, neuroimaging, and neurophysiologic features were obtained.

Results

A cohort of 142 patients was included. Characteristics of the *PURA* syndrome included neonatal hypotonia, feeding difficulties, and respiratory distress. Sixty percent of the patients developed epilepsy with myoclonic, generalized tonic-clonic, focal seizures, and/or epileptic spasms. EEG showed generalized, multifocal, or focal epileptic abnormalities. Lennox-Gastaut was the most common epilepsy syndrome. Drug refractoriness was common: 33.3% achieved seizure freedom. We found 97 pathogenic variants in *PURA* without any clear genotype-phenotype associations.

Discussion

The *PURA* syndrome presents with a developmental and epileptic encephalopathy with characteristics recognizable from neonatal age, which should prompt genetic screening. Sixty percent have drug-resistant epilepsy with focal or generalized seizures. We collected more than 90 pathogenic variants without observing overt genotype-phenotype associations.

Purine-rich element-binding protein A (*PURA*) gene, located on chromosome 5q31, encodes a single-exon transcript that results in a highly conserved 322 amino acid (AA) protein, Pur-α.[1] Pur-α is essential for normal postnatal brain development, as well as proliferation of neuronal cells and synapse formation.[2] Pur-α knock-out mice display a severe phenotype with neurologic dysfunction, including spontaneous seizures, tremor, and early death.[3] The Pur-$\alpha_{+/-}$ mouse shows hypotonia, gait deficits, and memory dysfunction, correlating with a loss of neurons in the cerebellum and hippocampus.[4]

Initially defined as the 5q31.3 microdeletion syndrome, after the discovery of point variants, the term *PURA* syndrome (OMIM #616158) was acknowledged, and more than 70 patients with pathogenic de novo variants in *PURA* have been published to date.[2,5-15] *PURA* phenotypes are heterogeneous and can include moderate to severe intellectual disability (ID), hypotonia, movement disorders, epilepsy, dysmorphic facial features, and brain abnormalities.[7,10] Most patients do not achieve independent walking and remain nonverbal.[7] The neonatal period is often complicated by respiratory insufficiency and feeding difficulties because of pronounced hypotonia, and children often have an exaggerated startle response.[7]

Epilepsy is reported in approximately half of the patients, with seizure onset usually in infancy—early childhood, although the age at onset has a wide range.[7] Seizure types are often refractory and are usually described as generalized tonic-clonic (GTC) seizures, absence of seizures, epileptic spasms, and tonic seizures, with the most commonly diagnosed epilepsy syndrome being Lennox-Gastaut syndrome.[7] Specific treatment recommendations are not currently available.

Genotype-phenotype correlations in *PURA* syndrome have been inconsistent so far, and even within patients with identical variants, phenotype and severity can vary significantly.[7]

In this study, we report on 142 patients including 67 unpublished cases aiming to expand and further define the phenotypic and genotypic spectrum of the *PURA* syndrome, with particular focus on the epilepsy phenotype.

Methods

Patients

Patients were recruited from genetic and epilepsy clinics worldwide. Most of the patients were referred through the *PURA*

Syndrome Foundation. Genetic variants were assessed for pathogenicity according to the American College of Medical Genetics and Genomics guidelines,[16] including criteria such as being nonsynonymous, splice-site altering, nonsense, or frameshift changes, predicted damaging by 1 or more prediction software (Poly-Phen-2, SIFT, and MutationTaster), not seen in controls in the GnomAD browser and occurring de novo. Pathogenic and likely pathogenic variants were included. Deletions were not included. Sanger sequencing was used to confirm all variants and perform segregation analysis.

Clinical characteristics were assessed through a standardized phenotyping sheet and included data on neuroimaging and neurophysiology, as well as treatment response. Treatment response was assessed by the referring clinician as seizure free (no seizure for >6 months at the time of the referral of the patient to the study), seizure reduction, no effect or seizure aggravation. Seizures were classified according to the latest proposals of the International League Against Epilepsy commission on classification.[17,18] EEG data were collected by means of standard EEG recording or daytime video-polygraphic recording, including wakefulness and sleep. Longitudinal EEG evaluation over time was not performed because of lack of data. Whenever possible, original EEG studies were evaluated by 2 neurologists with EEG expertise (E.G. and G.R.).

A PubMed search using the search term "PURA" was performed to extract data on previously published patients. Last search date: June 1, 2020.

Statistical Analysis

Clinical data were analyzed using Stata version 15.1 for Mac (StataCorp, College Station, TX). For continuous unmatched data, the Wilcoxon rank-sum (Mann-Whitney) was used. Significance was tested using a 2-tailed test of proportions, and significance was reached if $p < 0.05$. The do-file used to perform the analysis is available on request.

Standard Protocol Approvals, Registrations, and Patient Consents

The local ethical committees approved this study. All patients or parents/legal guardians in case of minors signed informed consent.

Data Availability

Those eligible will be granted access to deidentified patient data in excel format, as well as do-files to Stata on request. Data will be stored for 6 months after publication. Those eligible includes independent researcher wishing to perform additional data analysis.

Results

Sixty-seven previously unpublished patients were included along with 75 previously published patients—142 patients in total. Age at inclusion ranged from 5 months to 48 years (median 6 years). The total cohort is given in eTables 1 and 2 (links.lww.com/NXG/A448 and links.lww.com/NXG/A449).

Clinical Features

Neonatal Period

Data were available in 134 patients (94.4%); 114 of the 134 patients (85.2%) had congenital hypotonia and 72 of 134 (53.5%) had respiratory difficulties, the most common being central sleep apneas, which required mechanical ventilation or supplementary oxygen in 19 of the 72 patients (26.9%). Feeding difficulty was another common issue seen in 109 of the 134 patients (81.3%), and it required gastrointestinal tube feeding in 42 of the 109 patients (38.5%). Other features of the neonatal period included hypersomnolence (35/134, 26.1%), and hypothermia (15/134, 11.3%).

Childhood and Early Adulthood (<25 Years of Age)

Developmental milestones were delayed in all patients (analyzed in previously unpublished patients only, Table 1). At the time of inclusion, 47 patients achieved head control (70.1%), median age 16 months. Forty-three patients were able to sit (56.6%), median age 17.5 months. Thirty-four were able to walk with aid (50.7%), and 25 were walking independently (37.3%), median ages 5 and 3.5 years, respectively. Five patients regressed from walking to being nonambulatory. Communication skills were very limited in all the patients; 93.5% were nonverbal.

In childhood and early adulthood, hypotonia continued to be an overt clinical feature affecting 107 of the 134 patients (79.9%) with *PURA* syndrome. Movement disorders such as dyskinesia, hand stereotypies, ataxia, and chorea-like movements were detected in 32 of the 134 patients (23.9%). A pathologic startle response was present in 24 of 134 (17.9%). Additional common disorders were strabismus (28, 20.9%), horizontal nystagmus not related to antiseizure medication (ASM) (22, 16.4%), and cortical vision impairment, 15 (11.2%). In 8 of the 50 patients (16.0%) without epilepsy, episodes with twitching or with staring, not associated with any EEG correlate, were considered of nonepileptic nature.

Other common characteristics included constipation (27/134, 20.1%), scoliosis (26/134, 19.4%), hip dysplasia (21/134, 15.7%), cardiac and large vessel abnormalities (15/134,

Table 1 Age of Achievement of Developmental Milestones in the *PURA* Syndrome

Developmental milestones	Achieved in n patients (%)	Median age of achievement (range)
Head control	47 (70.1%)	16 mo (8–24 mo)
Rolling over	44 (65.7%)	13.5 mo (6–18 mo)
Sitting	43 (56.6%)	17.5 mo (10 mo–3.5 y)
Crawling	37 (55.2%)	2.5 y (1.5–4 y)
Walking with assistance	34 (50.7%)	5 y (2.5–10 y)
Walking	25 (37.3%)	3 y (1 y 4 mo–12 y)

Abbreviation: PURA = purine-rich element-binding protein A.

11.2%), dysphagia (15/134, 11.20%), delayed puberty (13/134, 9.7%), and small stature (12/134, 9.0%).

Adulthood (>25 Years of Age)

Nine patients were 25 years of age or older at the time of inclusion. All 9 patients had hypotonia. Three were capable of walking autonomously, 1 could walk with aid, and the remainder were nonambulant. All, but one, were nonverbal. Six had severe ID, while 3 had moderate ID.

Early Mortality

In our cohort, 4 patients were deceased: 1 (#39) died at the age of 15 years because of sudden unexpected death in epilepsy (SUDEP), whereas 1 (#40) died at the age of 3 years and 2 (#70 and #104) died in their twenties, in a state of severe general compromise after a relentless deterioration of neurologic conditions, ultimately because of respiratory distress.[5,7] Of notice, 2 of the patients (#39 and #40) carry the same variant, i.e., c.812_814del, p.(Phe271del). Two additional patients with this variant have been published (#108[8] and #109[7]); however, at the time of publication, both patients were very young (<1 year), so the disease course was still unknown. A summary of the clinical features is available in Table 2.

Table 2 Clinical Features of the Total *PURA* Cohort

Clinical features	
Sex, female/male (%)	93/64 (59.2%/40.8%)
Age at inclusion, median (range)	6 y (5 mo–48 y)
Epilepsy (%)	84 (59.2%)
Age of epilepsy onset, median (range)	3 y (1 d–18 y)
Most common seizure types (%)	Myoclonic (22.5%) GTC (21.3%) Focal (20.2%)
Neurologic features	n (%)
Neonatal period	
Hypotonia	121 (85.2%)
Feeding difficulties	115 (81.0%)
Respiratory distress	76 (53.5%)
Hypersomnolence	37 (26.1%)
Childhood to adulthood	
Hypotonia	113 (79.6%)
Strabismus	30 (21.1%)
Nonepileptic episodes (twitching or staring)	23 (16.2%)
Pathologic startle response	25 (17.6%)
Movement disorder	34 (23.9%)
Dysmorphic features	92 (64.8%)
Death	4 (2.8%)

Abbreviation: PURA = purine-rich element-binding protein A.

Neuroimaging

Half (51.4%) of the patients showed changes in the MRIs. Most commonly, these changes included delayed myelination (38.5%) and volume loss (14.1%).

Dysmorphology

Ninety-two patients (64.8%) displayed dysmorphic features. Facial photographs are shown in Figure 1. Subtle but recurrent similarities were observed including a hypotonic face, high anterior hairline, almond-shaped palpebral fissures, and full cheeks. A flat nasal bridge with a wide and triangular nasal tip, thickened nostrils, a well-defined philtrum, heavy eyebrows, and periorbital fullness was present in a subset of individuals.

Epilepsy Phenotype

Eighty-four patients (59.2%) had epilepsy. The median age at seizure onset was 3 years, ranging from the first day of life to 18 years. Age at inclusion in the subgroup of patients with epilepsy was 9.6 years (vs 4.9 years in the subgroup without epilepsy, $p = 0.0001$). Data on seizure types were available for 72 of the 84 patients (85.7%). Heterogeneous seizure types were reported (Figure 1), with the most common being myoclonic seizures (22.5%), GTC seizures (21.3%), focal seizures (20.2%) with secondary generalization (15.7%), epileptic spasms (19.1%), and atonic (14.6%) and tonic seizures (12.4%). Reflex seizures to auditory, visual, and somatosensory stimuli (such as touch, tooth brushing or hair brushing, pain, associated with evacuation) were reported in 6 patients (7.9%) (#17, #20, #31, #35, #91, and #92, eTables 1 and 2, links.lww.com/NXG/A448 and links.lww.com/NXG/A449).[8,9] Reflex seizures were described as myoclonic or atonic seizures, or "absence-like" episodes, and in some patients, they occurred for a transitory period (#31). Overall, half (53.7%) of the patients presented with various seizure types in different combinations, including either focal or generalized (such as absences, myoclonic, and GTC) seizure types in the same patient. A diagnosis of Lennox-Gastaut syndrome or West syndrome was made in 5 patients (#79, #92, #97, #99, and #100) and 2 patients (#19 and #40), respectively.[5,7,8] We attempted to investigate whether early-onset (<12 years) and later-onset (>12 years) epilepsies presented with different phenotypes. These analyses were hampered by the unbalanced distribution of the available information in the 2 groups because seizure types and age at onset were reported in 64 patients with onset before or at the age of 12 years and in 6 patients with onset after the age of 12 years. Four of the 6 patients with epilepsy onset after 12 years presented with focal, focal and secondary GTC, or GTC. Patients with onset before the age of 12 years presented with heterogeneous seizure types in variable combinations.

EEG

EEG data were collected in 104 of the 142 patients (73.2%), including in 67 of the 84 patients (79.8%) with epilepsy. Abnormal EEGs, not further specified, were reported in 30 epileptic participants (44.7%), whereas in 3 (4.4%), the EEG was described as unremarkable. In the remaining 37 patients, background slowing was reported in 19 of 37 (51.3%), whereas epileptic abnormalities were described as diffuse/generalized in 12 of the 37 patients (32.4%), multifocal in 10 of the 37 patients (27%), and focal in 7 of

Figure 1 The Faces of the *PURA* Syndrome

Faces of patients with pathogenic *PURA* variants. Recurrent similarities include a myopathic face, high anterior hairline, almond-shaped palpebral fissures, and full cheeks. A flat nasal bridge with a wide and triangular nasal tip, thickened nostrils, a well-defined philtrum, heavy eyebrows, and periorbital fullness was seen in a subset of individuals. PURA = purine-rich element-binding protein A.

the 37 patients (18.9%), respectively. Four children (10.8%) presented with an excessive activation of the epileptic abnormalities during sleep (resembling an electrical status epilepticus during sleep [ESES] pattern), whereas hypsarrhythmia and burst suppression were observed in 3 patients (8.1%) each (Figure 2).

EEG recordings were available also in 37 of the 57 patients (64.9%) without epilepsy. It showed poorly organized/slowed background activity in 8 of the 37 patients (21.6%), diffuse spikes in 1 of 37 (2.7%), and focal spikes in 2 of 37 (5.4%); in the remaining patients, it was diagnosed as abnormal not further specified in 1 of 37 (2.7%) and unremarkable in 25 of 37 (67.5%). At the time of the patient collection, none of the patient without epilepsy featuring a previously abnormal EEG was reported to have presented with epilepsy in the follow-up.

Epilepsy Treatment

Treatment response was evaluated in the patients with epilepsy from the previously unpublished cohort only (42 patients) because data were too sparse in the published patients.

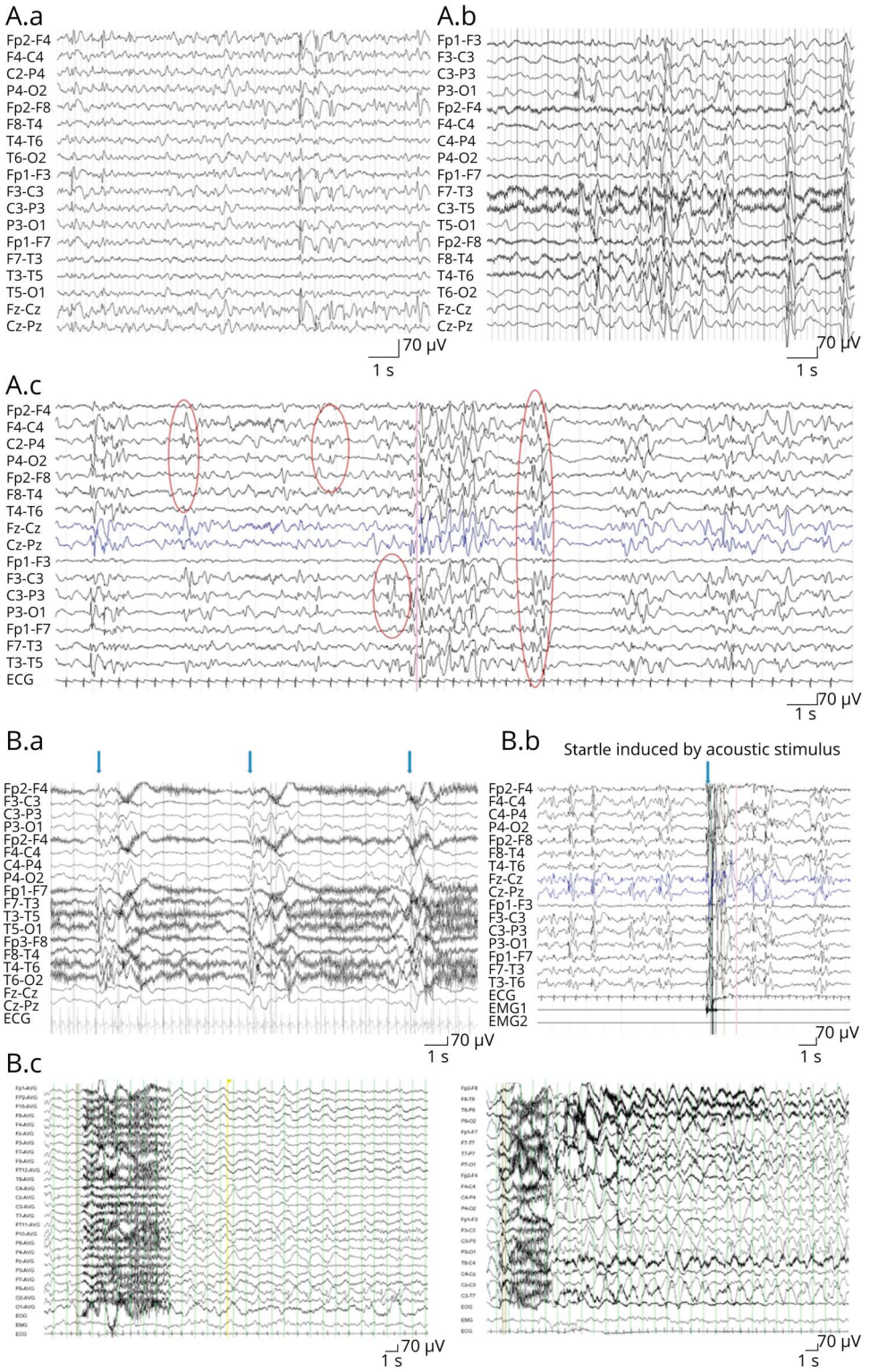
Most of the patients (28/42, 66.7%) were still having seizures despite appropriate treatment with ASM. Two patients were able to taper off monotherapy ASM (#36 and #41, age 18 years and 3 years 9 months) with sustained seizure freedom. Seizure freedom was achieved with valproate (VPA) in 4 patients, topiramate (TPM) in 3 patients, and levetiracetam (LEV) in 2 patients, either as monotherapy or in combination with other drugs. Seizure freedom was sustained for 3 to 8 years (median 3.8 years) at the time of inclusion of these patients in the study. Seizure reduction was observed with VPA in 8 patients, LEV in 5, TPM in 5, and lamotrigine in 4 patients. Seizure worsening or side effects causing cessation of drug use was seen with LEV in 3 patients, with VPA and TPM in 2 patients each, and with lacosamide in 1 patient, either as monotherapy or in combination with other drugs.

Six patients tried the ketogenic diet: 2 had a seizure reduction, while the remaining 4 had no effect. Cannabidiol was tried only in 2 patients, without any effect. Vagal nerve stimulation implantation was tried in 3 patients and resulted in seizure freedom in 1 patient and reduced seizure frequency in 2 patients.

Genetics

Ninety-seven different variants were identified in 142 patients; 38 were previously unpublished. In all cases where inheritance could be determined, variants occurred de novo. Missense variants were present in 24.6% of the patients, a 3-AA in-frame deletion was seen in 9.9%, while the remaining 65.5% carried

Figure 2 EEG Changes in the *PURA* Syndrome



(A) The interictal EEG is characterized by slow background and multifocal spike/sharp and slow waves predominant either in the frontal regions (a, pt. #42) or in the posterior quadrants (b, pt. #30). The epileptiform activity is accentuated during sleep; this image (c, pt. #19) shows very frequent multifocal spike/polyspikes and waves and sharp and slow waves, independently in the right central, right parietal, and left central regions or diffuse. (B) The ictal EEG showed (a) cluster of epileptic spasms (each arrow corresponds to a spasm) (pt. #30), (b) a startle induced by acoustic stimulus (pt. #19), and (c) brief tonic seizures out of sleep with an EEG correlation consisting of diffuse rapid activity (3–6 seconds) followed by diffuse delta activity and trains of spike and slow waves in the posterior regions (pt. #30). PURA = purine-rich element-binding protein A.

protein-truncating variants (PTVs) such as in-frame deletions, stop-, and frameshift variants. Missense variants and PTVs were seen in patients with similar phenotypes and both with and without epilepsy.

Nineteen variants were seen at the same position—most of them detected only in 2 or 3 patients, but the p.(Leu54Alafs*)/p.(Leu54Cysfs*) was seen in 6 patients, the p.(Val226Glyfs*)/p.(Val226Serfs*) was seen in 4 patients, the p.(Phe233del) was

seen in 14 patients, the p.(Arg245*)/p.(Arg245Pro) was seen in 4 patients, and the p.(Phe271del) was also seen in 4 patients (eTable 3, links.lww.com/NXG/A450). Patients both with and without epilepsy were seen in several of these variants, even within the same variant. See eTable 3 for a summary.

The variants were distributed throughout the gene: 33.1% were located in the PUR-I repeat, 21.8% in the PUR-II repeat, and 23.2% in the PUR-III repeat, whereas the remainder were outside the PUR repeats (Figure 3). Five missense variants were located outside the PUR repeats.[2,5,7]

Two patients had compound heterozygous variants (#62 and #97); however, their clinical presentation did not vary from the heterozygous variants,[5] inheritance was either de novo or unknown, and only 1 of the variants in each patient was defined as pathogenic according to the American College of Medical Genetics and Genomics guidelines.[16]

Discussion

With this comprehensive study of 142 patients, we provide an outline of the phenotypic and genotypic spectrum of the *PURA* syndrome and we significantly expand the current patient population, suggesting that pathogenic variants in *PURA* might underlie ID and developmental and epileptic encephalopathy (DEE), more often than previously thought.

This study was done retrospectively and through clinician assessment of patients. When original EEG tracings were not available for review, EEG evaluation was based on EEG reports, discussing them, when necessary, with the referring physician. Unfortunately, longitudinal EEG data were not available; therefore, it has not been possible to evaluate the EEG evolution during the course of the disease.

Some data points are missing in some patients, although follow-up with clinicians was attempted. Patients were also assessed only once for inclusion; thus, longitudinal clinical data were not available in this study.

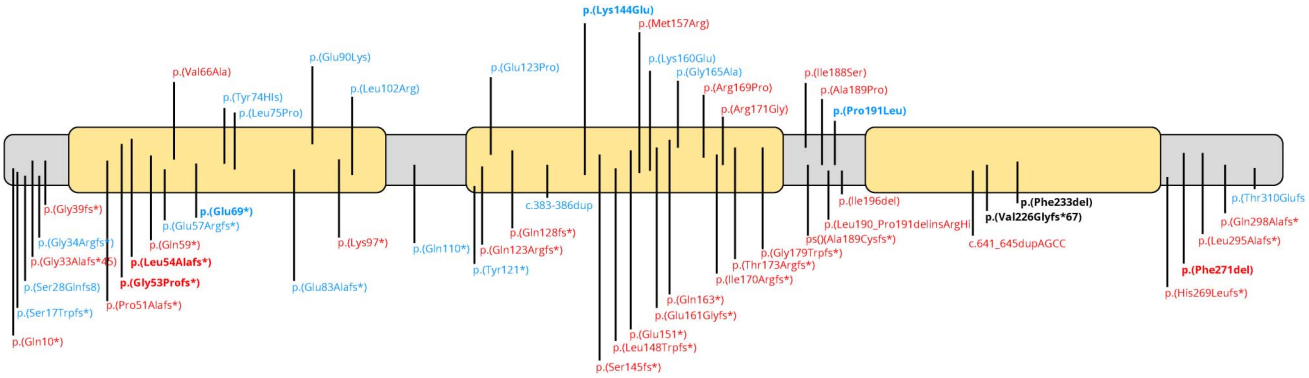
Most commonly, patients with *PURA* syndrome present with neonatal hypotonia, complicated by feeding difficulties, often requiring tube feeding, and respiratory distress.

The respiratory distress can be as severe as to cause respiratory insufficiency, requiring frequent recurrent pulmonary aspiration and often demanding mechanical ventilation because of the increased risk of central apnea, similar to the picture reported in patients with 5q31 microdeletions encompassing the *PURA* gene, who also feature a marked hypotonia at birth.[8]

Hypersomnolence and hypothermia further demonstrate the severity of the *PURA* syndrome. There were no significant differences between patients with and without neonatal hypotonia at inclusion, neither in the phenotypic appearances nor in the genetics. The hypotonia persists through childhood and adulthood, although in some patients, it gradually improves and motor skills continue to develop, eventually resulting in the acquisition of autonomous walking. This may reflect the steady increase of Pur-α during development, as seen in the mice.[3,19] The median age at inclusion was 6 years; thus, the estimated numbers are to be considered minimum values. In patients able to ambulate, gait is often ataxic; this finding can correlate with the fact that *PURA* expression peaks in the postnatal period, when interneuronal connections are being established, especially in the cerebellum.[3] Gait disturbances were also found in the heterozygous mouse.[4]

Nonepileptic paroxysmal motor manifestations and pathologic startles were reported in a significant proportion of patients, both with and without epilepsy. In recent years, the occurrence of nonepileptic movement disorders in participants with DEE has been increasingly recognized.[20,21] Indeed, hyperkinetic movement manifestations, such as dystonia and choreoathetosis, are now considered a common phenotypic feature in individuals

Figure 3 The *PURA* Gene



The *PURA* gene with previously unpublished variants. Missense on top, protein-truncating variants below. With epilepsy in red, without epilepsy in blue, and recurrent variant in bold. PUR repeats indicated by yellow coloring: PUR-I position 42–106, PUR-II position 120–182, and PUR-III position 197–252. PURA = purine-rich element-binding protein A.

with *FOXG1, GNAO1, SCN8A, CACNA1B,* and *STXBP1*-related epilepsy-dyskinesia syndromes.[22-26] Hand stereotypies are commonly observed in children with autism or developmental delay, irrespective of precise etiology,[27] and "per se" they do not confer any specificity to affected patients; however, the definition of detailed accompanying phenotypes (such as hyperkinetic movements or hand stereotypies) can be important to characterize the disease spectrum and the early recognition of the *PURA*-related DEE, ultimately prompting referral of the patients for genetic testing.

A pathologic startle was observed in approximately 18% of our patients. At variance with our patients, the Pur-$\alpha_{+/-}$ mouse shows a trend toward a reduced startle response to acoustic stimuli, a finding considered unexpected by the authors of the study[4] that requires to be further investigated, while the mouse also displayed occasional seizures on handling.[3] Exaggerated startle attacks, described as sudden tonic postures with raising of bent arms over the head, flexion of the neck and trunk, in response to touch or sound stimuli, not associated with epileptic changes in the EEG,[28] have been reported in 38% of a cohort of children presenting with an encephalopathy associated with *KCNQ2* pathogenic variants.[29] Our findings of a pathologic startle, previously unreported, might add to the constellation of clinical features that could contribute to an early diagnosis of *PURA*-related DEE.

In older patients, scoliosis is common because of truncal hypotonia. Other features include impaired intestinal motility (reflux and constipation most commonly), visual disturbances, and in a few patients hearing deficits as well. The underlying mechanisms for these features are unknown.

In our study cohort, 3 patients died because of respiratory failure and 1 because of SUDEP.[5,7,30] *PURA* knockout (KO) mice do not survive beyond 28 postnatal days[3]; however, an increased death rate is not observed in the heterozygous mice (*PURA*$_{+/-}$).[3] Thus, we can speculate that if the human phenotype is comparable with the heterozygous mouse, the mortality seen in our cohort might be an expression of the increased mortality associated with severe developmental disorders and epilepsy in general and not linked directly to *PURA*. On the contrary, if the mortality rate observed here is indeed related to *PURA*-related DEE, it is lower than that reported in Dravet syndrome (15.84/1,000 person-years),[31] whereas it is comparable with the mortality seen in other DEEs, such as *SCN8A*-related DEE (mortality 5.3%), which also shows similar causes of death.[32] Lack of longitudinal follow-up in this study is a limitation in the assessment of the mortality. Further studies, in particular investigating the natural history of the *PURA*-related DEE, might be helpful to solve this issue and to establish the mortality rate of this DEE.

MRIs were normal in half of the patients, whereas the other half showed demyelination or loss of brain volume. In 1 patient (#54), follow-up MRI showed a normalization of myelination over time; therefore, we could speculate that myelination in patients with *PURA* syndrome is not absent but delayed. Further follow-up MRI data in additional patients will clarify this issue. Experimental studies in KO mouse have provided conflicting results, showing in some reports decreased signals from myelin and white matter tracts both in the subcortical white matter and in the cortex compared with wild-type littermates and reduced neuron density both in the cortex and in the cerebellum.[3] Other studies in KO mice showed an enlarged brain size associated with a phenotype showing ataxia and continuous tremor.[33] The finding of a megalocephalic brain has been interpreted as resulting from a prolongation of neuronal precursor cell proliferation during postnatal development, an explanation that further supports the role of Purα in postnatal brain development.[3]

In a computational analysis of photographs of 34 *PURA* syndrome individuals, it was found that affected patients shared a similar gestalt, including a hypotonic face and high anterior hairline.[7] In addition to those features, we expand the facial gestalt to include a flat nasal bridge with a widened and triangular nasal tip, thickened nostrils, a well-defined philtrum, heavy eyebrows, and periorbital fullness, present in a subset of individuals. Many syndromes have recognizable facial features that are highly informative to clinical geneticists.[34-36] Although *PURA* is not an easily recognizable syndrome, a detailed description of dysmorphic features and an understanding of how the features change over time can help clinicians identify further cases.

In total, 59.2% of the patients analyzed in this study have epilepsy, with age at onset ranging from the first day of life to 18 years. In the heterozygous mouse model, seizures/epilepsy are constant features in all animals.[3] Most of the patients presented with a combination of seizure types that could be both focal and generalized in the same patient. Our results show that the most common seizure types are myoclonic, GTC, and focal with secondary generalization. A not negligible group of patients presented with reflex seizures whose semiology includes myoclonic, atonic, and "absence-like" manifestations. Various stimulus types (including visual, auditory, sensory, and painful stimulations), were reported as effective in triggering seizures. In some patients, a reflex mechanisms was observed only for a transitory period; therefore, we cannot exclude that the occurrence of reflex seizures in patients with *PURA* syndrome might be underestimated, particularly in patients with severe ID, high seizure frequency, co-occurrence of nonepileptic paroxysmal motor disorders, and limited monitoring.

The EEG also showed a mixture of focal/multifocal and generalized epileptic abnormalities. Of interest, an excessive activation of EEG epileptic discharges during sleep, resembling an ESES EEG pattern, was reported in 4 children; whether this sleep activation was associated with further cognitive/behavioral deterioration as in ESES syndrome was not possible to demonstrate because of the severity of the baseline cognitive status. Our findings suggest that *PURA* might add to the growing list of DEE-associated genes that

can present during the course of the disease with an ESES-like EEG pattern.[37,38] Hypsarrhythmia and burst suppression were rarely observed. A syndromic classification that considered both the clinical and EEG features was possible only in 6 patients, with 4 of them presenting a clinical picture compatible with a Lennox-Gastaut syndrome and the other 2 with West syndrome. The remaining patients with epilepsy could be broadly diagnosed as presenting with a DEE, in most cases with a severe course.

In total, 40.9% of patients in our cohort did not present with epilepsy. However, because of the broad age range of epilepsy onset (from the first day of life up to 18 years) and the age at inclusion in this study, only 1 patient without epilepsy was older than 18 years. Thus, we cannot exclude that a proportion of nonepileptic patients will develop epilepsy later in the course of their disease, in particular those nonepileptic patients who already present epileptic abnormalities in their EEG. This hypothesis may be supported by the statistical significant difference in the age at inclusion in our study of the subgroup of patients with epilepsy who were older than the subgroup without epilepsy. The nonepileptic patient older than 18 years was retrieved in the literature, and he was a 19-year-old man who presented with developmental delay since birth; he was able to walk by the age of 4 years and to communicate with a one-two word vocabulary.[13] He had a normal MRI and normal EEG (the last one at 14 years of age).[13] He carried a de novo recurrent missense variant, p.(Ile188Thr), as well as an Xp22.31 duplication.[13] In the literature, another patient with the same missense variant also had no seizure activity at the age of 8 years,[2] whereas a third patient with a frameshift variant at the same position had GTCs with an unknown age at onset.[5] Thus, at present, an estimation of the prevalence of epilepsy in the *PURA* population is not reliable because most of the reported patients are children, who could still present with epilepsy at a later age. In line with this, pediatric epilepsy cohort studies have not identified *PURA* as one of the more common causes of epilepsy in infancy.[39] However, in a recent study from our center, we identified a pathogenic variant in *PURA* as the underlying cause of epilepsy in 1% of adult patients.[40]

Our results show that 66.6% of the patients with *PURA* syndrome suffer from drug-refractory epilepsy. Although some ASMs, such as VPA, LEV, and TPM, showed favorable results in some patients, the same drugs were ineffective in other patients, currently making treatment recommendations for patients with *PURA* syndrome difficult. Further studies elucidating the underlying pathophysiologic mechanisms in *PURA*-related epilepsy will be crucial, including (heterozygous) animal models to test possible novel drugs and to identify possible precision medicine approaches.

In this study, we expand the genetic landscape of *PURA* by including 38 new variants. In all patients where segregation analysis was completed, the variants arose de novo. In addition, most of the patients have variants that are loss of function per se, such as frameshift or indel variants. This is supported by a pLoF (predicted loss-of-function) score of 8.9 (pLi 0.94), suggesting that the *PURA* gene is intolerant to loss of function variants (gnomad.broadinstitute.org/). Thus, haploinsufficiency seems to be the most likely functional defect underlying the disease. Although missense variants have not been tested functionally, the evidence that patients with missense variants display phenotypes similar to the patients with loss of function variants points toward similar functional effects.

Most of the variants were located within the PUR repeats, which underline the importance of these repeats for the function of *PURA*.[41] Some variants, both nonsense and missense, are still located outside the PUR repeats, suggesting that these areas of the gene are also crucial for the correct functioning of *PURA*.

Phenotypic variability was seen even within variants; where the same variant was seen in patients with and without epilepsy. This might reflect other factors, such as epigenetic modulators, or it might be simply due to a difference in age of the patients (see above).

Previously, a single patient with a variant in the C-terminal of the protein was described with a milder phenotype compared with other patients, which led the authors to suggest that it is unlikely for variants in the far end of the C-terminal to affect protein folding or nucleic acid binding.[7] However, our results do not support this hypothesis because also patients with variants in this part of the gene (such as #42) seem to display an equally severe *PURA* phenotype. There were also no apparent differences between variants in the 5′ and 3′ end of the protein. No clear genotype-phenotype associations emerged from the analysis of our cohort.

Our study further defines and expands the phenotypic and genotypic spectrum of the *PURA*-related DEE. Some clinical features, especially in the neonatal age, including hypotonia, feeding, and respiratory difficulties, are characteristic and should raise the suspicion of *PURA*, prompting genetic screening. In our cohort, more than 60% of patients have epilepsy, characterized by a very wide range of age at onset, from the neonatal period up to early adulthood; therefore, it cannot be excluded that a further fraction of patients, still in their childhood and not presenting epilepsy yet, will develop epilepsy later in their disease course. The epilepsy featured a wide spectrum of seizure types, including both focal, generalized seizures, and reflex seizures, refractory to currently available ASMs in the great majority of patients. Finally, our study failed to identify genotype-phenotype associations. Additional studies investigating the functions of *PURA* and the pathophysiologic mechanism underlying the *PURA*-related DEE are needed to guide the search for more effective and possibly targeted treatments.

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Disclosure

J. Buratti has worked as a consultant for Alexion, Audentes, AveXis, Biogen, Cytokinetics, Genentech, Momenta, PTC Therapeutics, Sarepta, and WaVe. The other authors report no disclosures. Go to Neurology.org/NG for full disclosures.

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Appendix *(continued)*

Name	Location	Contribution
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Appendix *(continued)*

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Continued

Appendix *(continued)*

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Felicia Varsalone, MD	Pediatric Neurology Unit, V. Buzzi Childrens Hospital, 20154 Milan, Italy	Major role in the acquisition of data
Sabine Grønborg, MD	Department of Neuropediatrics, University Hospital Copenhagen, Copenhagen, Denmark	Major role in the acquisition of data
Cyril Mignot, MD, PhD	APHP Trousseau, Paris, France	Major role in the acquisition of data

Appendix *(continued)*

Name	Location	Contribution
Delphine Heron, MD	APHP Trousseau, Paris, France	Major role in the acquisition of data
Caroline Nava, MD, PhD	Department of Genetics, Pitié-Salpêtrière hospital, APHP, Sorbonne Université, Paris, France	Major role in the acquisition of data
Arnaud Isapof, MD	Service de Neuropédiatrie, Hopital Trousseau, Sorbonne Université, APHP.SU, Paris, France	Major role in the acquisition of data
Felippe Borlot, MD	Alberta Childrens Hospital, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada; Division of Neurology, Department of Paediatrics, The Hospital for Sick Children, Toronto, Ontario, Canada	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Robyn Whitney, MD	Division of Neurology, Department of Paediatrics, The Hospital for Sick Children, Toronto, Ontario, Canada	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Anne Ronan, MD	Hunter Genetics Unit, Waratah, Australia	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Nicola Foulds, MD	Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton, United Kingdom	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Marta Somorai, MD	KBO Kinderzentrum Munchen, Munich, Germany	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
John Brandsema, MD	Division of Neurology, Epilepsy Neurogenetics Initiative, Childrens Hospital of Philadelphia; Perelman School of Medicine, Philadelphia, PA	Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data
Katherine L. Helbig, MSc	Division of Neurology, Epilepsy Neurogenetics Initiative, Childrens Hospital of Philadelphia, PA	Major role in the acquisition of data
Ingo Helbig, MD, PhD	Division of Neurology, Epilepsy Neurogenetics Initiative, Childrens Hospital of Philadelphia, PA; PURA Syndrome Foundation, Greensborough, Australia; PURA Syndrome Foundation, Kansas City, MO	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data; Obtaining funding
Xilma R. Ortiz-González, MD	Division of Neurology, Epilepsy Neurogenetics Initiative, Childrens Hospital of Philadelphia, PA	Major role in the acquisition of data

Continued

Appendix *(continued)*

Name	Location	Contribution
Holly Dubbs, MD	Division of Neurology, Epilepsy Neurogenetics Initiative, Childrens Hospital of Philadelphia, PA	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Antonio Vitobello, PhD	INSERM UMR1231, GAD team, Universit´e de Bourgogne-Franche Comt´e, Dijon, France; Unit´e Fonctionnelle dInnovation diagnostique des maladies rares, Pole de Biologie, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France	Analysis or interpretation of data; Obtaining funding
Mel Anderson, MSc	PURA Syndrome Foundation, Greensborough, Australia	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data
Dominic Spadafore, MSc	PURA Syndrome Foundation, Greensborough, Australia; PURA Syndrome Foundation, Kansas City, MO	Major role in the acquisition of data
David Hunt, MD	Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton, United Kingdom	Major role in the acquisition of data
Rikke S. Møller, MSc, PhD	Department of Epilepsy Genetics and Personalized Treatment, The Danish Epilepsy Centre Filadelfia, Dianalund, Denmark; Institute for Regional Health Research, University of Southern Denmark, Odense, Denmark	Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data
Guido Rubboli, MD, PhD	The Danish Epilepsy Center Filadelfia/University of Copenhagen, Dianalund, Denmark	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data; Study supervision or coordination

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