PURA-Related Developmental and Epileptic Encephalopathy
Phenotypic and Genotypic Spectrum

Katrine M. Johansen, MD, PhD, Elena Gardella, MD, PhD, Catherine E. Gjerulfsen, MD, Allan Bayat, MD, Rob P.W. Roulh, MD, Margot Reijnders, MD, Sandra Whalen, MD, Boris Keren, MD, PhD, Julien Buratti, MSc, Thomas Courtin, MD, Klaas J. Wierenga, MD, Bertrand Isidor, MD, Amélie Piton, MD, Laurence Faivre, MD, Aurore Garde, MD, Sébastien Mouton, MD, Frédéric Tran-Mau-Them, MD, Anne-Sophie Denommée-Pichon, MD, Christine Coubes, MD, Austin Larson, MD, Michael J. Esser, MD, Juan Pablo Appendino, MD, Valia Al-Hertani, MD, Beatriz Gamboni, MD, Alexandra Mampel, MD, Lia Mayorga, MD, PhD, Alessandro Orsini, MD, PhD, Alice Bonuccelli, MD, Agnese Suppiej, MD, Julien Van-Gils, MD, Julie Vogt, MD, Simona Damioli, MD, Lucio Giordano, MD, Stephanie Moortgat, MD, PhD, Elaine Wirrell, MD, Sarah Hicks, MD, Usha Kini, MD, Nathan Noble, DO, Helen Stewart, MD, Shailesh Asakar, MD, Julie S. Cohen, MD, Sakkubai R. Naidu, MD, Ashley Collier, MD, Eva H. Brilstra, MD, Mindy H. Li, MD, Casey Brewe, MD, Stefania Bignoni, MD, Davide Ognibene, MD, Elisia Ballardini, MD, Claudia Ruvenkamp, MD, Raffaella Faggioni, MD, Alexandra Ajenfarj, MD, Diana Rodriguez, MD, David Bick, MD, Devdip Seth, MD, PhD, David Coman, MD, Boudewijn Gunnin, MD, Onrin Devinsky, MD, Lautaro A. Trujillo, MD, Theresa Grebe, MD, Carla Proni, MD, Ida Guroso, MD, Lynn Greenlee, MD, MSc, Claudio Graziano, MD, PhD, Rahul Raman Singh, MD, Gaetano Cantalupo, MD, Marjolaine Willems, MD, Sangeetha Yoganathan, MD, Fernando Goeis, MD, Richard J. Leverentz, MD, PhD, Davide Colavito, MD, Sara Olivotto, MD, Barbara Scelsa, MD, Andrea V. Andrade, MD, Kelly Ratke, PhD, Farha Tokarz, MD, Atiya S. Khan, MD, Clothilde Ormeiras, MD, William Benko, MD, Karen Keough, MD, Sotirios Keros, MD, Shanawaz Hussain, MD, Ashley Franques, MD, Felicia Varsalone, MD, Sabine Granborg, MD, Cyril Mignot, MD, PhD, Delphine Heron, MD, Caroline Nava, MD, PhD, Arnaud Isapo, MD, Felippe Borlot, MD, Robyn Shrubsole, MD, Anne Ronan, MD, Nicola Foulds, MD, Marta Somorai, MD, John Brandsma, MD, Katherine L. Helbig, MSc, Ingo Helbig, MD, PhD, Xilma R. Ortiz-González, MD, Holly Dubbs, MD, Antonio Vitobello, PhD, Mel Anderson, MSc, Dominic Spadafore, MSc, David Hunt, MD, Rikke S. Møller, MSc, PhD, Guido Rubboli, MD, PhD,* and the PURA study group

Neural Genet 2021;7:e613. doi:10.1212/NXG.0000000000000613

*Guido Rubboli, MD, PhD, is designated as the last author of this article.

From the Department of Epilepsy Genetics and Personalized Treatment (K.M.), E.G., A.B., R.S.M., G.R.), The Danish Epilepsy Centre Filadelfia, member of ERN EpiCARE, Dianalund; Institute for Regional Health Research (K.M.), E.G., A.B., R.S.M., G.R.), University of Southern Denmark, Odense; Department of Neurology (R.P.W.R.), Maastricht University Medical Centre (MUMC+); Academic Centre for Epileptology Kempenhaeghe/MUMC+ (R.P.W.R.), Maastricht; School for Mental Health and Neuroscience (R.P.W.R.), Maastricht University; Department of Clinical Genetics (M.R.), Maastricht University Medical Center, the Netherlands; APHP, Sorbonne University (S.W.), Hôpital Armand Trousseau, UFR de Généthique Clinique, Centre de Référence Anomalies du Développement et Syndromes Malformatifs, Paris, France; Department of Genetics (B.K., J.B., T.C., C.N.), Pitie-Salpêtrière hospital, APHP, Sorbonne University, Paris, France; Department of Clinical Genomics (K.J.W.), Mayo Clinic, Florida, Jacksonville; Service de Génétique Médicale (I.L., A.P., M.J.), CHU de Nantes; Centre de Référence Anomalies du Développement et Syndromes Malformatifs (L.F., A.G.M.), Hôpital Universitaire (F.T.M.-T., A.V.), CHU de Dijon; Uniforce Fonctionnelle dionnaisique diagnostiche des maladies rares (F.T.-M.-T., A.V.), Pôle de Biologie, H9U-TRANSALD, CHU Dijon Bourgogne; Department of Medical Genetics (C.C., M.W.), Rare Diseases and Personalized Medicine, CHU Montpellier; France; ANR-15-CE11-0043, “Ann acht Campus Medical, Aurora, CO; Division of Clinical Neuroscience (M.R.), Department of Paediatrics, Alberta Children’s Hospital (P.A., F.B.), Cumming School of Medicine, University of Calgary, Alberta, Canada; Department of Neurology (P.A.-W.H.), Division of Genetics and Genomics, Boston Children’s Hospital and Harvard Medical School, MA; Instituto de Neurologia Infantil Juvenil (B.G.), Neuroinfan; Instituto de Genética Hospital Universitario (A.M.), Universidad Nacional de Cuyo; Instituto de Estadística y Biomecánica de Mendoza (HEM) (L.M.), Universidad Nacional de Cuyo, Mendoza, Argentina; Azienza Ospedaliera Universitaria Pisana (A.P.), Neurosurgical Section (A.B.), Pediatric Department; Santa Chiara University Hospital, Pisa; Department of Medical Sciences- Pediatric Section (A.S.), University of Ferrara, Italy; CHU Bordeaux (I.V.-G.), Bordeaux, France; West Midlands Regional Genetics Service (I.V.), Birmingham Women’s and Children’s Hospital, Birmingham, UK; Child Neuropsychiatric Research Group (S.D., L.G.), PedsQOL, Brescia, Italy; Institute de Pathologie et de Génétique (P.I.G.) (S.M.), Gosselies, Belgium; Division of Child and Adolescent Neurology and Epilepsy (E.W.), Department of Neurology, Mayo Clinic, Rochester, MN; Oxford Centre for Genomic Medicine (S.H., H.S.); Oxford University Hospitals NHS Trust (UK); United Kingdom; Blank Children’s Developmental Center (N.N.), Unity Point Health, West Des Moines, IA; Sutter Medical Centre (S.A.), Sacramento, CA; Kennedy Krieger Institute (J.S.C.), Johns Hopkins University (S.R.N.), Baltimore, MD; Provincial Medical Genetics Program (A.C.), St. Johns Medical Center, NL, Canada; University Medical Center Utrecht (E.B.H.), Utrecht, the Netherlands; Rush University Medical Center (M.L.H., C.B.), Chicago, IL; Medical Genetic Unit (B.D., D.O.), Maternal and Child Development, Ferrara University Hospital; Medical Science Department (D.O.), Ferrara University; Neonatal Intensive Care Unit (E.B.), Pediatric Section, Medical Science Department, Ferrara University Hospital, Medical Science Department (D.O.), Ferrara University; Department of Paediatrics, Ferrara University Hospital; University of Verona, Italy; Christian Medical College (S.Y.), Vellore, India; Neurology Pediatric Unit (F.G.), Pediatric Department, Federneau Figueras Institute; Fiscuz, Brazil; Royal Children Hospital (F.J.L.), Melbourne, Australia; Research & Innovation Srl (D.C.), Padova; Pediatric Neurology Unit (G.O., B.S., F.V.), V. Buzzi Children’s Hospital, Milan, Italy; Department of Paediatrics (A.V.A.), London Health Science Centre/Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada; Ambiy Genetic (K.R.), Aliso Viejo, CA; Advocate Lutheran General Hospital (F.T.), Park Ridge, IL; PPG Pediatric Neurology (A.S.K.), Parkview Health, Fort Wayne, IN; Department of Medical Genetics (C.O.), AP-HP, Necker-Enfants Malades Hospital, Paris, France; Department of Neurology (W.B.), UC Davis, Sacramento, CA; Department of Pediatrics (K.K.), Texas A&M University Medical School, Austin; Leeds General Infirmary (S.H.), United Kingdom; Thompson River Pediatrics (A.F.), Johnston, CO; Department of Paediatrics (S.G.), University of Liverpool, Copenhagen, Denmark; Division of Neurology (F.B., R.W.), Department of Paediatrics, The Hospital for Sick Children, Toronto, Ontario, Canada; Hunter Genetics Unit, Warahah, Australia (A.R.); Weesies Clinical Genetics Service, Princess Anne Hospital, Southampton, United Kingdom (N.F., D.H.); KBO-Kinderzentrum München, Munich, Germany (M.S.); Division of Neurology (S.G.), Epilepsy Neurogenetics Initiative, Children’s Hospital of Philadelphia (J.B., X.H., I., X.R.O.-G., H.D.), Perlman School of Medicine, Philadelphia, PA (J.B.); PURA Syndrome Foundation, Greensborough, Australia (I.H., M.A., D.S.); PURA Syndrome Foundation, Kansas City, MO (I.H., D.S.);

Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by The PURA Syndrome Foundation.

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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.

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.

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. Pur-α is essential for normal postnatal brain development, as well as proliferation of neuronal cells and synapse formation. Pur-α knock-out mice display a severe phenotype with neurologic dysfunction, including spontaneous seizures, tremor, and early death. The Pur-α+/− mouse shows hypotonia, gait deficits, and memory dysfunction, correlating with a loss of neurons in the cerebellum and hippocampus.

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. PURA phenotypes are heterogeneous and can include moderate to severe intellectual disability (ID), hypotonia, movement disorders, epilepsy, dysmorphic facial features, and brain abnormalities. Most patients do not achieve independent walking and remain nonverbal. The neonatal period is often complicated by respiratory insufficiency and feeding difficulties because of pronounced hypotonia, and children often have an exaggerated startle response.

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. 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. 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.

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, 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. 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, 11.3%).

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. Of notice, 2 of the patients (#39 and #40) carry the same variant, i.e., c.812_814delPhe271del. Two additional patients with this variant have been published (#108 and #109); 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 (%)                |
| Respiratory distress                          | 76 (53.5%)          |
| Hypotonia                                     | 121 (85.2%)         |
| Feeding difficulties                          | 115 (81.0%)         |
| Hypersomnolence                               | 37 (26.1%)          |
| Strabismus                                    | 30 (21.1%)          |
| Nonepileptic episodes (twitching or staring)   | 23 (16.2%)          |
| 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). Reflex seizures were described as myoclonic or tonic 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. 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...
the 37 patients (18.9%), respectively. Four children (10.8%) pre-
sented with an excessive activation of the epileptic abnormalities
during sleep (resembling an electrical status epilepticus during
sleep \[ESES\] pattern), whereas hypsarhythmia and burst sup-
pression 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%), di-
ffuse 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 fur-
ther 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 epi-
lepsy from the previously unpublished cohort only (42 pa-
tients) 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 im-
plantation was tried in 3 patients and resulted in seizure free-
dom 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
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

**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) a cluster of epileptic spasms (each arrow corresponds to a spasm) (pt. #30), (b) a startle induced by acoustic stimulus (pt. #19), and (c) a brief tonic seizure 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.
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.5

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-a 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.5 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 choreathetosis, 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 STXBPI-related epilepsy-dyskinesia syndromes.22−26 Hand stereotypes 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 stereotypes) 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-a+/− mouse shows a trend toward a reduced startle response to acoustic stimuli, a finding considered unexpected by the authors of the study4 that requires to be further investigated, while the mouse also displayed occasional seizures on handling.5 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.5,7,34 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 megalencephalic 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 Pura 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 geneticists34−36 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, GTCS, 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 mechanism 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. 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. He had a normal MRI and normal EEG (the last one at 14 years of age). He carried a de novo recurrent missense variant, p.(Ile188Thr), as well as an Xp22.31 duplication. In the literature, another patient with the same missense variant also had no seizure activity at the age of 8 years, whereas a third patient with a frameshift variant at the same position had GTCs with an unknown age at onset. 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. 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.

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 PURA repeats, which underline the importance of these repeats for the function of PURA. Some variants, both nonsense and missense, are still located outside the PURA 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. 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.

Acknowledgment
The authors are deeply grateful for the collaboration and support from all the PURA families, and the authors wish to acknowledge the PURA foundation for their tireless work and commitment to advance the knowledge on PURA syndrome.
The authors thank Diana Baralle for inclusion of patients and Dierk Niessing and Robert Janowski for their invaluable support and comments on the manuscript. The authors thank the following for their contributions to the study: Deborah Mitchell-Langlois. The authors thank Jean-François Deleuze, Anne Boland-Augé, and Robert Olaso for the collaboration CEA/JACOB/CNRGH—CHU de Dijon—Inserm.

**Study Funding**

The open access for this paper was funded by the PURA Syndrome Foundation. J. Buratti received research funding as a site investigator from Alexion, AveXis, Biogen, CSL Behring, Cytokinetics, Fibrogen, Pfizer, PTC Therapeutics, Sarepta, Summit, and WaVe. I. Helbig was supported by The Hartwell Foundation through an Individual Biomedical Research Award. This work was also supported by the National Institute for Neurological Disorders and Stroke (K02 NS112600), including support through the Center Without Institute for Neurological Disorders and Stroke (K02 Award). This work was also supported by the National Foundation through an Individual Biomedical Research Summit, and WaVe. I. Helbig was supported by The Hartwell Foundation through an Individual Biomedical Research Award. This work was also supported by the National Institute for Neurological Disorders and Stroke (K02 NS112600), including support through the Center Without Institute for Neurological Disorders and Stroke (K02 Award). This work was also supported by the National Foundation through an Individual Biomedical Research Summit, and WaVe. I. Helbig was supported by The Hartwell Foundation through an Individual Biomedical Research Award. This work was also supported by the National Institute for Neurological Disorders and Stroke (K02 NS112600), including support through the Center Without Institute for Neurological Disorders and Stroke (K02 Award). This work was also supported by the National Foundation through an Individual Biomedical Research Summit, and WaVe. I. Helbig was supported by The Hartwell Foundation through an Individual Biomedical Research Award. This work was also supported by the National Institute for Neurological Disorders and Stroke (K02 NS112600), including support through the Center Without Institute for Neurological Disorders and Stroke (K02 Award).

**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.

**Publication History**

Received by Neurology: Genetics November 3, 2020. Accepted in final form June 29, 2021.

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**Appendix Authors**

| Name | Location | Contribution |
|------|----------|--------------|
| Katrine M. Johannesen, MD, 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/review 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 |
| Elena Gardella, MD, 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 | Major role in the acquisition of data |
| Cathrine E. Gjerulfsen, MD | 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 | Major role in the acquisition of data |
| Allan Bayat, MD | 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/review of the manuscript for content, including medical writing for content |
| Rob P.W. Rouhl, MD | Department of Neurology, Maastricht University Medical Centre (MUMC+), Maastricht, Netherlands Academic Centre for Epileptology Kempenhaeghe/MUMC+, Maastricht, Netherlands School for Mental Health and Neuroscience, Maastricht University, Maastricht, the Netherlands | Drafting/review of the manuscript for content, including medical writing for content; Major role in the acquisition of data |
| Margot Reijnders, MD | Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, the Netherlands | Major role in the acquisition of data |
| Sandra Whalen, MD | APHP.Sorbonne Université, Hôpital Armand Trousseau, UF de Génétique Clinique, Centre de Référence Anomalies du Développement et Syndromes Malformatifs, Paris, France | Major role in the acquisition of data |
| Boris Keren, MD, PhD | Department of Genetics, Pitie-Salpêtrière hospital, APHP, Sorbonne Université, Paris, France | Major role in the acquisition of data |
| Julien Buratti, MSc | Department of Genetics, Pitie-Salpêtrière hospital, APHP, Sorbonne Université, Paris, France | Major role in the acquisition of data |
| Thomas Courtin, MD | Department of Genetics, Pitie-Salpêtrière hospital, APHP, Sorbonne Université, Paris, France | Major role in the acquisition of data |
| Name                      | Location                                                                 | Contribution                                      |
|---------------------------|--------------------------------------------------------------------------|--------------------------------------------------|
| M. J. Wierenga, MD        | Department of Clinical Genomics, Mayo Clinic Florida, Jacksonville       | Major role in the acquisition of data            |
| Bertrand Isidor, MD       | Service de Génétique Médicale, CHU de Nantes, France                     | Major role in the acquisition of data            |
| Amélie Piton, MD          | Service de Génétique Médicale, CHU de Nantes, France                     | Major role in the acquisition of data            |
| Laurence Faire, MD        | Centre de Référence Anomalies du Développement et Syndromes Malformatifs, FHU TRANSLAD, CHU Dijon, Dijon, France; INSERM UMR1231, GAD team, Université de Bourgogne-Franche Comité, Dijon, France | Major role in the acquisition of data |
| Aurore Garde, MD          | Centre de Référence Anomalies du Développement et Syndromes Malformatifs, FHU TRANSLAD, CHU Dijon, Dijon, France; INSERM UMR1231, GAD team, Université de Bourgogne-Franche Comité, Dijon, France | Major role in the acquisition of data |
| Sébastien Moutton, MD     | Centre de Référence Anomalies du Développement et Syndromes Malformatifs, FHU TRANSLAD, CHU Dijon, Dijon, France; INSERM UMR1231, GAD team, Université de Bourgogne-Franche Comité, Dijon, France | Major role in the acquisition of data |
| Frédéric Tran-Mau-Them, MD| INSERM UMR1231, GAD team, Université de Bourgogne-Franche Comité, Dijon, France; Unité Fonctionnelle dinnovation diagnostique des maladies rares, Pôle de Biologie, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France | Major role in the acquisition of data |
| Anne-Sophie Denommé-Pichon, MD | Service de Génétique Médicale, CHU de Nantes, France                  | Study concept or design                          |
| Christine Coubes, MD      | Department of Medical Genetics, Rare Diseases and Personalized Medicine, CHU Montpellier, Montpellier, France | Major role in the acquisition of data |
| Austin Larson, MD         | Childrens Hospital Colorado, Anschutz Medical Campus, Aurora, CO        | Major role in the acquisition of data            |
| Michael J. Esser, MD      | Division of Clinical Neuroscience, Department of Pediatrics, Alberta, Canada | Major role in the acquisition of data            |
| Juan Pablo Appendino, MD  | Division of Clinical Neuroscience, Department of Pediatrics, Alberta, Canada; Alberta Childrens Hospital, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada | Major role in the acquisition of data |
| Walla Al-Hertani, MD      | Department of Pediatrics, Division of Genetics and Genomics, Boston Childrens Hospital and Harvard Medical School, Boston, MA | Major role in the acquisition of data |
| Beatriz Gamboni, MD       | Instituto de Neurología Infanto Juvenil, Neuroinfan, Mendoza, Argentina | Major role in the acquisition of data |
| Alejandro Mampel, MD      | Instituto de Genetica-Hospital Universitario, Universidad Nacional de Cuyo, Mendoza, Argentina | Major role in the acquisition of data |
| Lia Mayorga, MD, PhD      | Instituto de Histologia y Embriología de Mendoza (IHEM), Universidad Nacional de Cuyo, Mendoza, Argentina | Major role in the acquisition of data |
| Alessandro Orsini, MD, PhD| Azienda Ospedaliera Universitaria Pisana, Pisa, Italy                  | Major role in the acquisition of data            |
| Alice Bonuccelli, MD      | Neuropaediatric Section, Pediatric Department, Santa Chiara University Hospital, Pisa, Italy | Major role in the acquisition of data |
| Agnese Suppiej, MD        | Department of Medical Sciences-Pediatric Section, University of Ferrara, Ferrara, Italy | Major role in the acquisition of data |
| Julien Van-Gils, MD       | CHU Bordeaux, Bordeaux, France                                           | Major role in the acquisition of data            |
| Julie Vogt, MD            | West Midlands Regional Genetics Service, Birmingham Women's and Children's Hospital, Birmingham, UK | Major role in the acquisition of data |
| Simona Damioli, MD        | Child Neuropsychiatric Division, Spedali Civili, Brescia, Italy          | Major role in the acquisition of data            |
| Lucio Giordano, MD        | Child Neuropsychiatric Division, Spedali Civili, Brescia, Italy          | Major role in the acquisition of data            |
| Stephanie Moortgat, MD, PhD| Institut de Pathologie et de Génétique (IPG), Gosselies, Belgium         | Major role in the acquisition of data            |
| Elaine Wirrell, MD        | Divisions of Child and Adolescent Neurology and Epilepsy, Department of Neurology, Mayo Clinic, Rochester, MN | Major role in the acquisition of data |
| Sarah Hicks, MD           | Oxford Centre for Genomic Medicine, Oxford, United Kingdom               | Major role in the acquisition of data            |

Continued
| Name                            | Location | Contribution                      |
|---------------------------------|----------|-----------------------------------|
| Usha Kini, MD                   | Oxford University Hospitals NHS Trust, Oxford, United Kingdom | Major role in the acquisition of data |
| Nathan Noble, DO               | Blank Children's Developmental Center, Unity Point Health, West Des Moines, IA | Major role in the acquisition of data |
| Helen Stewart, MD              | Oxford Centre for Genomic Medicine, Oxford, United Kingdom | Major role in the acquisition of data |
| Shailesh Asakar, MD            | Sutter Medical Centre, Sacramento, CA | Major role in the acquisition of data |
| Julie S. Cohen, MD             | Kennedy Krieger Institute, Baltimore, MD | Major role in the acquisition of data |
| SakkuBai R. Naidu, MD          | Johns Hopkins University, Baltimore, MD | Major role in the acquisition of data |
| Ashley Collier, MD             | Provincial Medical Genetics Program, St. Johns Medical Center, NL, Canada | Major role in the acquisition of data |
| Eva H. Brilstra, MD            | University Medical Center Utrecht, Utrecht; the Netherlands | Major role in the acquisition of data |
| Mindy H. Li, MD                | Rush University Medical Center, Chicago, IL | Major role in the acquisition of data |
| Casey Brew, MD                 | Rush University Medical Center, Chicago, IL | Major role in the acquisition of data |
| Stefania Bigoni, MD            | Medical Genetic Unit, Maternal and Child Dept, Ferrara University Hospital, Ferrara, Italy | Major role in the acquisition of data |
| Davide Ognibenene, MD          | Medical Genetic Unit, Maternal and Child Dept, Ferrara University Hospital, Ferrara, Italy; Medical Science Dept, Ferrara University, Ferrara, Italy | Major role in the acquisition of data |
| Elisa Ballardini, MD           | Neonatal Intensive Care Unit, Pediatric Section, Department of Medical Sciences, Ferrara University, Ferrara, Italy | Major role in the acquisition of data |
| Claudia Ruivenkamp, MD         | Department of Clinical Genetics, LUMC, Leiden, the Netherlands | Major role in the acquisition of data |
| Raffaella Faggioni, MD         | Pediatric Unit, Maternal and Child Dept, Ferrara University Hospital, Ferrara, Italy | Major role in the acquisition of data |
| Alexandra Afenjar, MD          | APHP Trousseau, Paris, France | Major role in the acquisition of data |
| Diana Rodriguez, MD            | Service de Neuropédiatrie, Hopital Trousseau, Sorbonne Université, APHP-SU, Paris, France | Major role in the acquisition of data |
| David Bick, MD                 | HudsonAlpha Institute for Biotechnology, Huntsville, AL | Major role in the acquisition of data |
### Appendix (continued)

| Name                  | Location                                                                 | Contribution                                                                 |
|-----------------------|--------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Davide Colavito, MD   | Research & Innovation S.r.l., Padova, Italy                               | Major role in the acquisition of data                                         |
| Sara Olivotto, MD     | Pediatric Neurology Unit, V. Buzzi Childrens Hospital, Milan, Italy       | Major role in the acquisition of data                                         |
| Barbara Scelsa, MD    | Pediatric Neurology Unit, V. Buzzi Childrens Hospital, Milan, Italy       | Major role in the acquisition of data                                         |
| Andrea V. Andrade, MD | Department of Paediatrics, London Health Science Centre/Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada | Major role in the acquisition of data                                         |
| Kelly Ratke, PhD      | Ambry Genetics, Aliso Viejo, CA                                          | Major role in the acquisition of data                                         |
| Farha Tokarz, MD      | Advocate Lutheran General Hospital, Park Ridge, IL                       | Major role in the acquisition of data                                         |
| Atiya S. Khan, MD     | PPG Pediatric Neurology, Parkview Health, Fort Wayne, IN                 | Major role in the acquisition of data                                         |
| Clothilde Ormieres, MD| Department of Medical Genetics, AP-HP, Necker-Enfants Malades Hospital, Paris, France | Major role in the acquisition of data                                         |
| William Benko, MD     | Department of Neurology, UC Davis, Sacramento, CA                        | Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data |
| Karen Keough, MD      | Department of Pediatrics, Texas A&M University Medical School, Austin    | Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data |
| Sotirios Keros, MD    | Department of Pediatrics, Weill Cornell Medicine, New York, NY          | Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data |
| Shanawaz Hussain, MD  | Leeds General Infirmary, Leeds, United Kingdom                           | Major role in the acquisition of data                                         |
| Ashlea Franques, MD   | Thompson River Pediatrics, Johnstown, CO                                  | Major role in the acquisition of data                                         |
| Felicia Varsalone, MD | Pediatric Neurology Unit, V. Buzzi Childrens Hospital, 20154 Milan, Italy | Major role in the acquisition of data                                         |
| Sabine Grenborg, 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                                         |
| 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, Hospital 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 Munich, 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, Children's Hospital of Philadelphia, PA | Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data |
| Antonio Vitello, PhD| INSERM UMR1231, GAD team, Université de Bourgogne-Franche Comté, INRA, Dijon, France; Unité de Fonctionnelle d'Innervation diagnostique des maladies rares, Pole de Biologie, FHMU-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 |
| Dominican 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; Study concept or design; Study supervision or coordination |

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Neurol Genet 2021;7;
DOI 10.1212/NXG.0000000000000613

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