PURA syndrome: clinical delineation and genotype-phenotype study in 32 individuals with review of published literature

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ABSTRACT

Background *De novo* mutations in *PURA* have recently been described to cause PURA syndrome, a neurodevelopmental disorder characterized by severe intellectual disability (ID), epilepsy, feeding difficulties and neonatal hypotonia.

Objectives To delineate the clinical spectrum of PURA syndrome and study genotype-phenotype correlations.
Methods Diagnostic or research-based Exome or Sanger sequencing was performed in individuals with ID. We systematically collected clinical and mutation data on newly ascertained *PURA* syndrome individuals, evaluated data of previously reported individuals, and performed a computational analysis of photographs. We classified mutations based on predicted effect using 3D *in silico* models of crystal structures of *Drosophila*-derived Pur-alpha homologues. Finally, we explored genotype-phenotype correlations by analysis of both recurrent mutations as well as mutation classes.

Results We report mutations in *PURA* in 32 individuals, the largest cohort described so far. Evaluation of clinical data, including 22 previously published cases, revealed that all have moderate to severe ID and neonatal-onset symptoms, including hypotonia (96%), respiratory problems (57%), feeding difficulties (77%), exaggerated startle response (44%), hypersomnolence (66%) and hypothermia (35%). Epilepsy (54%), gastro-intestinal- (69%), ophthalmological- (51%), and endocrine problems (42%) were observed frequently. Computational analysis of facial photographs showed subtle facial dysmorphism. No strong genotype-phenotype correlation was identified by subgrouping mutations into functional classes.

Conclusion We delineate the clinical spectrum of PURA syndrome with the identification of 32 additional individuals. The identification of one individual through targeted Sanger sequencing points towards the clinical recognizability of the syndrome. Genotype-phenotype analysis showed no significant correlation between mutation classes and disease severity.

Keywords: PURA syndrome, intellectual disability, hypotonia, seizures, neonatal problems

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INTRODUCTION

Deletions in the 5q31.2q31.3 region have been reported to cause a syndrome comprising severe developmental delay, profound hypotonia, feeding difficulties, abnormal breathing pattern and seizures.[1-5] Within this region, haploinsufficiency of the purine-rich element binding protein A (PURA) has been suggested as responsible for the observed neurodevelopmental features by narrowing the region of overlap to three genes.[4] Further evidence for the essential role of PURA in development, came from two articles describing fifteen individuals with intellectual disability (ID) and *de novo* mutations in *PURA*.[6, 7] The pathogenicity of mutations in *PURA* was further supported by publication of a cohort of six additional individuals with ID and *de novo* mutations in *PURA* and two case reports.[8-10]

The functionality of PURA is dependent on three PUR motifs, PUR I, PUR II and PUR III.[11, 12] The ubiquitously expressed PURA protein, Pur-alpha, has multiple regulatory functions in processes such as DNA replication and transcription, mRNA transport, and DNA repair.[12, 13] Several studies have shown that Pur-alpha has an important role in neuronal development and differentiation.[14] In animal models, Pur-alpha is involved in postnatal brain development.[15, 16] The Pur-alpha deficient knockout mouse model has a severe neurological phenotype, including tremor and spontaneous seizures.[16] Recently, heterozygous *PURA* knock-out mice have been reported to develop gait abnormalities, hypotonia and memory deficits. Immunohistochemical assays displayed a reduced number of neurons in cerebellum and hippocampus of these animals, which is in line with their neurological, behavioural and cognitive phenotypes.[17]

Mental Retardation, Autosomal Dominant Type 31[MIM616158], or 'PURA syndrome', the name of the syndrome adopted by the parents and clinical community alike, is phenotypically hallmarked by a wide spectrum of neurodevelopmental problems, including severe neurodevelopmental delay, epilepsy, abnormal movements, hypotonia and brain abnormalities. Additionally, neonatal respiratory insufficiency and feeding difficulties have been reported. Outside of this core phenotype, a broad variability in clinical severity has been observed within PURA syndrome.[6-9] Different types of mutations have been identified so far and mutations occur throughout the gene. It has been suggested that disruption of PUR repeat III possibly results in a more severe phenotype, but additional studies are needed to better understand the genotype-phenotype correlation of *PURA* mutations.[7]

We present 32 individuals with *de novo* mutations in *PURA*. With this large cohort, we delineate the clinical spectrum of PURA syndrome. Computational modeling shows that subtle overlapping facial dysmorphism is present. Additionally, we present an approach for genotype-phenotype analysis of identified mutations and associated phenotypes, revealing that observed phenotypic variability is probably not associated with type and localization of mutations.

METHODS

Identification of PURA syndrome individuals and collection of clinical and mutational data

Whole Exome Sequencing (WES) was performed in individuals with intellectual disability (ID) in either diagnostic (n=20) or research (n=10) settings (Supplementary Methods). In one individual targeted massively parallel sequencing (MPS) of a severe childhood epilepsy gene panel was used and in a second individual, clinically suspected to have PURA syndrome, *PURA* [NM_005859] was investigated with targeted Sanger sequencing. Parental samples were tested to assess *de novo* state. A questionnaire with clinical and mutational details was completed by clinicians. Written consent for publication of photographs was obtained from legal guardians.

Evaluation of previously published cases

Twenty-two individuals with *PURA* mutations have been described in four different publications.[6-9] We used the same questionnaire, to collect clinical and mutation data from individuals (n=22) reported in the literature.

Computational analysis of facial photographs

To visualize the characteristic facial features of PURA syndrome individuals, we generated a realistic, de-identified average based on 34 photographs of individuals (in this paper presented and previously published photographs) at different ages. A control average was created from 301 age-matched, healthy individuals. We used a fully-automated, algorithm that (1) annotated a constellation of 68 facial landmarks points on the face, (2) created an average face mesh and (3) warped faces of each individual onto the average face mesh. The face averaging algorithm was inspired by previous work[18, 19] and improved upon with better de-identification of individuals (Supplementary methods). Interpretation of the composite PURA face was performed by a panel of five independent dysmorphologists.

Functional assessment of *PURA* mutations in structural homology models

For homology modeling of mutations, the crystal structures of the N-terminal PUR domain (PDB-ID: 3K44) and of the C-terminal PUR domain (PDB-ID: 5FGO) from *Drosophila melanogaster* were used as template. Homology structures of human PUR domains were calculated using the PHYRE2 program.[20] In the case of the C-terminal PUR domain, the sequences of two repeat IIIs were merged into one peptide chain to allow for modeling of the entire domain. The human homology models showed high confidence scores and were used for *in silico* mutational analyses using the program Coot.[21] All mutations identified in our cohort and previously published cohorts[6-9] were included. Variants in *PURA* reported in the Exome Aggregation Consortium (ExAC) database[22] were included as controls. Deletions, frameshift, nonsense, and missense mutations were manually classified depending on the position, size and function of the affected amino acids. Point mutations were *in silico* introduced using the standard rotamer library of Coot and analysed for the appearance of steric clashes or repulsive forces of side chains. They were classified based on the orientation of mutated side chains and on our knowledge of functionally important surface regions. Figures were prepared using the program Pymol (www.pymol.org).

RESULTS

Identification of 32 individuals

We report 32 individuals with mutations in *PURA* (Figure 1). All, but two, were identified using WES in a diagnostic setting (individuals 1-10, 20-27, 30 and 32) or research setting (individuals 12-19, 29 and 31). Individual 28 was identified using targeted MPS of a severe childhood epilepsy gene panel. Individual 11 was clinically suspected to have PURA syndrome based on post-term birth, severe neonatal hypotonia, hypersomnolence, exaggerated startle, persistent apnoeic episodes requiring continuous SpO2 monitoring and supplementary oxygen, and feeding difficulties warranting NG tube placement. Targeted Sanger sequencing revealed a *PURA* mutation. Mutations were *de novo* in 29 individuals. For the remaining three individuals, no parental blood was available for testing. None of the individuals had a second, likely pathogenic, genetic variant in WES data. All identified missense mutations localized in one of the PUR repeat sequences (Figure 1).

Clinical delineation of 32 individuals

Clinical features observed in individuals reported in this study, are summarized in Table 1. Numbers and percentages of features were corrected for the number of individuals without available information on a specific feature.More extensive and detailed clinical information is available in Supplementary Table 1.

Table 1: Percentage of clinical features reported in individuals in this article (n=32) and meta-analysis with previously published PURA individuals (n=22) [6-9]

Clinical feature	**This article (n = max. 32)**		**Literature (n = max. 22)[6-9]**		**Total (n = max. 54)**	
	Percentage	Number	Percentage	Number	Percentage	Number
Growth						
Short stature (<-2.5 SD)	19%	5/27	14%	3/22	16%	8/49
Pregnancy/delivery						
Gestational age >41 weeks	56%	18/32	60%	3/5	57%	21/37
Neonatal problems						
Hypotonia	97%	31/32	94%	15/16	96%	46/48
Feeding difficulties	81%	25/31	73%	16/22	77%	41/53
GERD	28%	8/29	17%	1/6	26%	9/35
Breathing problems	48%	15/31	68%	15/22	57%	30/53
Hypersomnolence	66%	19/29	NR	NR	66%	19/29
Hypothermia	37%	10/27	25%	1/4	35%	11/31
Excessive hiccups in utero	55%	6/11	NR	NR	55%	6/11
Neurological abnormality						
Moderate-severe ID	100%	32/32	100%	22/22	100%	54/54
Hypotonia	97%	31/32	80%	4/5	95%	35/37
Stereotypic hand movements	36%	8/22	NR	NR	36%	8/22
Exaggerated startle response	58%	11/19	27%	4/15	44%	15/34
Epilepsy	50%	16/32	59%	13/22	54%	29/54
Delayed myelination	28%	9/32	24%	5/21	26%	14/53
Movement disorder	20%	6/30	30%	3/10	22%	9/40
Other brain abnormalities	31%	10/32	29%	6/21	30%	16/53
Skeletal abnormality						
Scoliosis	28%	9/32	18%	2/11	26%	11/43
Hip dysplasia	23%	7/31	0%	0/11	17%	7/42
Hyperlaxity	43%	9/21	9%	1/11	31%	10/32
Gastro-intestinal abnormality						
Constipation	62%	18/29	50%	3/6	60%	21/35
Drooling	66%	20/30	83%	5/6	69%	25/36
Respiratory abnormality	26%	8/31	27%	4/15	26%	12/46
Cardiac abnormality	13%	4/32	25%	2/8	15%	6/40
Urogenital abnormality	26%	8/31	9%	1/11	21%	9/42
Ophthalmological abnormality	40%	12/30	67%	14/21	51%	26/51
Endocrine abnormality						
Vitamin D deficiency	47%	7/15	25%	1/4	42%	8/19

Other	26%	5/19	50%	2/4	30%	7/23
Skin abnormality						
Soft skin	46%	6/13	NR	NR	46%	6/13

Abbreviations: GERD = gastroesophageal reflux disease; NR = not reported; ID = intellectual disability

Gestation and neonatal problems

More than half (56%) of individuals were born after > 41 weeks gestation. Induction of labour and/or caesarian section were reported in 14 individuals. In 55% (6/11), excessive hiccups were mentioned in utero. Neonatal problems were evident in all children immediately after birth. Hypotonia was present in all but one of the neonates, often causing feeding difficulties (81%). Apneas and congenital hypoventilation were reported in 48% of the neonates. Both feeding and respiratory problems often required monitoring in hospital: in 20 neonates, tube feeding, oxygen supplementation and/or mechanical ventilation were needed. Hypersomnolence (66%) and hypothermia (37%) were observed in a significant number of neonates. In about half of the neonates (58%) an exaggerated startle response was noted.

Development

All individuals with *PURA* mutations have moderate to severe intellectual disability with severe language and motor delay. Most individuals remain non-verbal (29/32, 91%), but many have better receptive language than expressive language and can follow simple instructions. The majority never achieved independent ambulation (17/31, 55%). For those who did, the age of first steps ranged from 28 months-7 years. Regression of achieved skills due to the onset of seizures has been reported in six individuals.

Neurologic abnormalities

A variety of neurological problems were observed in individuals with *PURA* mutations. Hypotonia, often more prominent in the trunk, was present from birth. Spasticity of extremities at older age was reported in three individuals, and Babinski response was reported in another two. Gait, if achieved, was often unstable and broad-based. Stereotypic hand movements, in some cases such as described for Rett syndrome[MIM312750], were observed in several individuals (36%). Six out of 30 (20%) were mentioned to have a movement disorder, including dystonia, chorea-like movements, seizure-like movements with normal EEG and ataxic movements. Delayed myelination is the most frequently reported brain abnormality observed on MRI images (28%). Other, mostly a-specific, observed brain abnormalities (in 31% individuals) include white matter abnormalities, prominent periventricular spaces, mild parenchymal atrophy, widening of lateral ventricles and underdeveloped rostrum of the corpus callosum. Peripheral neuropathy was measured in five individuals at young age (Supplemental Table 1).

Half of the individuals (50%) were diagnosed with epilepsy. For most of them, seizures started at the age of 2-4 years, but the age of onset ranged from 6 months to 15 years. Different seizure types were reported, including generalized tonic-clonic seizures, focal seizures, absence seizures, epileptic spasms, tonic seizures, drop attacks and over time, evolution to Lennox-Gastaut syndrome. The epilepsy is often refractory to medical treatment and some individuals have never been seizure-free. A longer follow up period will be required to determine the true prevalence of epilepsy, how refractory it is, and what treatments are most effective.

Ophthalmologic abnormalities

Eye abnormalities and visual problems were described frequently in PURA syndrome individuals (40%). Strabismus (often esotropia) was the most commonly reported finding along with strabismus-associated refractive errors (often hypermetropia). Cortical visual impairment was reported in three individuals; however, it is important to note that diagnostic criteria for this condition are highly variable. One individual was treated successfully for a congenital retinoblastoma. Nystagmus was also described in a small number of cases (4/29; 14%) but details of the phenotype are missing in reported cases.

Skeletal abnormalities

Progressive hip dysplasia and scoliosis were present in respectively 23% and 28% of the cases and could possibly be related to chronic truncal hypotonia (with or without abnormalities of limb tone), joint laxity and delayed or incomplete motor development. Surgical correction was required in five individuals in this cohort to date. The oldest individual was 18 years at time of surgery. Longer follow up might show a larger number of surgical interventions, since the majority of included individuals (27/32) had an age of examination below 18 years. Individuals showed a tendency to short stature with five having a height <-2,5 SD, and six others with a height between -2 SD and -2.5 SD.

From the remaining 16 individuals, only two had a height >0 SD. Low bone density (Z-score -4; 57%) was identified in a single case. Pes planus was present in 11 individuals.

Gastro-intestinal abnormalities

Due to severe hypotonia, swallowing problems and excessive drooling were observed in more than half of the individuals (66%). Constipation was often present (62%) and may be severe, requiring the use of laxatives from early age.

Endocrine abnormalities

A diverse range of endocrine problems were reported, with low vitamin D most commonly reported (47%), and less frequently observed abnormalities including aberrant sex hormone levels, abnormal cortisol response and aberrant thyroid hormone levels (26%) (Supplemental Table 1). It is likely that these problems are more common than observed, since not all individuals have been tested for endocrine abnormalities.

Congenital structural malformations

Although not frequently reported, some individuals had congenital malformations of the heart, urogenital- and/or respiratory tract. Cardiac malformations included a ventricular septal defect in two individuals, and an aberrant left subclavian artery, pulmonary stenosis and mild, spontaneously closed persistent ductus arteriosus each in one individual. Abnormalities of the urogenital tract were described in five individuals: cryptorchidism in three, kidney stones in two, and congenital hydronephrosis with megaureter and urinary reflux each in one individual. Laryngeal cleft was reported in a single case.

Facial dysmorphisms

Photographs of 21 individuals are shown in Figure 2. No striking similarities were observed, but a myopathic face, high anterior hairline, almond shaped palpebral fissures and full cheeks were reported recurrently by clinicians. The presence of these features was confirmed by a panel of five independent dysmorphologists, all being asked to analyze photographs of PURA syndrome individuals from figure 2. They also mentioned eversion of lower lateral eyelids, prominent, well defined philtrum and retrognathia as feature present in a subset of individuals.

Expansion of the cohort with 22 previously published cases

We structurally analyzed mutational and clinical data of 22 previously published individuals.[6-9] An overview of published mutations is present in Figure 1 and summarized clinical information is available in Supplementary Table 2.

Recurrence of mutations
We found four recurrent mutations: p.(Lys97Glu) in individual 20 and subject #4 in Lalani et al[6]; p.(Phe271del) in individual 28 and subject #1 in Lalani et al.[6]; p.(Arg245Pro) in individuals 1 and 15; p.(Phe233del) in individuals 4, 5 and 14, patient 4 in Tanaka et al[8] and patient 4 in Hunt et al.[7]. In addition, individuals 24 and 31 had two distinct mutations (c.153delA and c.155delG, respectively) that were both predicted to give rise to the same truncated protein product (p.(Leu54Cysfs*24)). There were six individuals between whom two distinct missense mutations were identified at each of three different amino acid positions: p.(Leu100Arg) and p.(Leu100Pro) in individual 21 and subject #1 in Lalani et al.[6]; p.(Ile206del) and p.(Ile206Phe) in individual 25 and patient 3 in Hunt et al.[7]; p.(Val226Serfs*68) and p.(Val226Glyfs*67) in individuals 6 and 10.

Clinical evaluation
Clinical features such as neurodevelopmental delay, epilepsy, brain- and ophthalmological abnormalities have been reported in all previously published cohorts. But for the majority of features, no clinical information is available across all previously published cohorts. For example, hypersomnolence, stereotypic hand movements and excessive hiccups in utero have not been reported in literature before. Using the summarized clinical information (Supplementary Table 2), we calculated the frequency of clinical features in all 54 individuals. (Table 1)

Computational analysis of facial dysmorphism

Since no striking overlap in facial dysmorphism was observed, but similarities between several individuals have been reported by clinicians, we performed an objective computational analysis on in this paper presented and previously published photographs, and compared it to the average image based on healthy controls (Figure 3). Ages at photographs ranged from 2 months to 19 years. A panel of five independent dysmorphologists agreed that a myopathic face with typically open mouth appearance and full cheeks were visible on the computational modeled PURA face. Additionally, two dysmorphologists reported a slightly abnormal shape of the eyes as (1) shorter palpebral fissures and (2) eversion of lower lateral eyelids. The high anterior hairline observed in a subset of individuals (6,7,8,9,11,13,14,16,17,28) was not visible on the computational model of the PURA face.

Modeling of all reported mutations into structural homology models of human Pur-alpha

Using the previously reported crystal structures of the N- and C-terminal PUR domains from the fruit fly *Drosophila melanogaster*[11, 12] as templates, we generated structural homology models for the corresponding domains of the human Pur-alpha paralog. Based on these homology-modeled structures all 46 reported mutations were analyzed and classified according to their predicted effects on domain folding and function.

The identified frameshift and nonsense mutations affecting the entire protein C-terminal to the site of mutation, were defined as Class A mutations. Depending on whether mutations occurred in the N-terminal PUR-domain and thus affect N- and C-terminal PUR domains or in the C-terminal PUR only affecting this very domain plus C-terminus, we subclassified them as A1 and A2 mutations, respectively (Figure 1B). Mutations that were predicted to affect folding only locally, for instance via amino acid exchange of a buried residue or short amino acid deletions in a folded region, were defined as Class B mutations (Figure 1C). Since the RNA- and DNA-binding mode of *Drosophila* Pur-alpha has been structurally analyzed[12], the human homology model could also be used to predict mutations that affect nucleic-acid binding. Such mutations were found in four individuals and were defined as Class C mutations (Figure 1D). We also found one mutation likely affecting a surface-exposed residue that was neither predicted to impair protein folding nor nucleic-acid binding. We suggest that this residue is involved in not-yet understood functions such as protein-protein interactions and we defined this as Class D mutation (Figure 1D). Finally, we classified one mutation as class E: p.(Met1?), causing loss of the start codon. Translation is therefore likely to start at the next inframe start codon, which is located at protein position 104. As a result, the protein will lack part of the N-terminal PUR domain, while it will have an intact C-terminal PUR domain (Figure 1E). As control we analyzed variants reported by ExAC, a database containing variants of healthy controls (Figure 1F). All of these variants resulted in

single amino acid exchanges that are either located outside the globular domains or were exchanged against residues with similar side-chain properties. Variant p.(Gln136Pro) is the only exception to this rule. It is located in the large loop region between repeat I and II and thus likely allows to compensate the changes in backbone conformation upon mutation into proline.

Genotype-phenotype analysis

Remarkable clinical variability has been observed within the total cohort of PURA syndrome individuals. To assess whether this variability could be explained by the type and/or localization of the identified mutations, we compared (1) phenotypes of individuals with the same mutation and (2) phenotypes of individuals with a mutation of the same class.

1. Recurrent mutations

We observed four recurrent mutations and two mutations with a similar protein effect within the cohort, and compared their phenotypes. For all mutations, remarkable differences between individuals with the same mutation were present (Table 2). For example, drug-resistant epilepsy was present in 3 out of 5 individuals with mutation p.(Phe233del) and a cardiac abnormality in 2 out of 5 individuals. The oldest of the two individuals without epilepsy could walk, in contrast to the three individuals with epilepsy, who were not able to walk or speak. For mutation p.(Leu54Cysfs*24), individuals had more features in common, such as epilepsy, scoliosis and short stature, but variation in ability to walk and heart abnormality was also observed.

Table 2: Recurrent PURA mutations and phenotype of affected individuals

Mutation	Individual/Age	Reference	Phenotype
p.(Leu54Cysfs*24)	Individual 24 17 years	This article	Hypotonia, feeding difficulties requiring TF, GERD, apneas, hypersomnolence, hypothermia, severe ID, no speech, walking at age 4 years, short stature (-5 SD), epilepsy, mild PDA, mild strabismus, scoliosis, hip dysplasia, small hands and feet.
	Individual 31 16 years	This article	Hypotonia, feeding difficulties requiring TF, hypersomnolence, severe ID, no speech, not able to walk, short stature (-2.75 SD), epilepsy, scoliosis, epilepsy, long fingers
p.(Lys97Glu)	Individual 20 2.5 years	This article	Hypotonia, mild feeding difficulties, hypersomnolence, no speech, not able to walk, mild constipation, pes planus
	Subject #4 21 months	Lalani et al [6]	Hypotonia, feeding difficulties, respiratory difficulties, seizures, ID, non-verbal, non-ambulatory, short stature (1%), strabismus, duplex left kidney, hydronephrosis
p.(Phe233del)	Individual 4 14 years	This article	Hypotonia, feeding difficulties, apneas, hypersomnolence, severe ID, no speech, able to walk, ASD, stereotypic hand movements, delayed myelination, swallow problems, severe hypermetropia, strabismus, low vitamin D, low ferritin, pes planus, abnormal peripheral nerve testing
	Individual 5 19 years	This article	Hypotonia, feeding difficulties, apneas, hypersomnolence, hypothermia, severe ID, no speech, not able to walk, stereotypic hand movements, epilepsy, nystagmus, delayed myelination, drooling, constipation, strabismus, CVI, scoliosis, hip dysplasia, low bone mineralization, low vitamin D, anemia, delayed puberty, small hands, pes planus
	Individual 14 9 years	This article	Hypotonia, severe ID, no speech, first steps age 7 years, epilepsy, delayed myelination, aberrant left subclavian artery, VSD, drooling, refraction abnormality, strabismus, low vitamin D, high cholesterol
	Patient 4 6 months	Tanaka et al [8]	Hypotonia, CVI, Periventricular leukomalacia
	Patient 4 6 years, 9 months	Hunt et al [7]	Hypotonia, feeding difficulties requiring TF, apneas, hypothermia, severe ID, not able to walk, essentially non-verbal, exaggerated startle response, dystonia, dyskinesia, epilepsy, CVI, delayed myelination, excessive extra-axial fluid spaces, cerebral atrophy, hyperprolactinemia, blunted cortisol stress response, low vitamin D
p.(Arg245Pro)	Individual 1 19 years	This article	Hypotonia, feeding difficulties, severe ID, no speech, not able to walk, autistic-like traits, chorea-like movements, Babinski

			response, delayed myelination, scoliosis, low vitamin D, hypogonadotropic hypogonadism
	Individual 15 9 years	This article	Hypotonia, feeding difficulties requiring TF, apneas, hypersomnolence, hypothermia, severe ID, no speech, first steps at age 4 years, epilepsy, drooling, constipation, pes planus
p.(Phe271del)	Individual 28 11 months	This article	Hypotonia, neonatal convulsions, moderate ID
	Subject #1 6 months	Lalani et al [6]	Hypotonia, feeding difficulties, respiratory difficulties, seizures, ID, pedal edema

Abbreviations: ASD = Autism Spectrum Disorder; CVI = Cortical Visual Impairment; GERD = Gastro Esophageal Reflux Disease; ID = Intellectual Disability; TF = Tube Feeding

2. Comparison of mutation classes

We compared phenotypes of individuals subdivided by mutation class. (Supplemental Table 4) Overall, no significant differences in percentage of observed features between mutation classes were present. One exception is mutation p.(Glu283Argfs*45), which we classified as class D. This mutation affects only the very C-terminus (all three PUR domains are intact) and it is unlikely that this mutation interrupts protein folding or nucleic-acid binding (Supplemental Table 3; Figure 1). Clinical features in this individual are less severe than observed in other individuals: the 14-years old girl could walk independently from the age of 2 years and could speak in sentences. Furthermore, no neonatal problems, hypotonia, epilepsy or brain abnormalities were present.[7] The clinical presentation of this girl is remarkably milder than other individuals, and could possibly be explained by the localization of the mutation at the C-terminus.

DISCUSSION

We report 32 individuals with PURA syndrome, the largest cohort so far. We systematically collected mutation and clinical data on these and previously published individuals. With information on a total of 54 individuals, we were able to delineate the clinical spectrum of this recently discovered syndrome.

The detailed and systematic collection of phenotypic data, allowed us to observe less frequently reported features related to PURA syndrome. Clinical problems such as excessive hiccups in utero (55% in our cohort), hypersomnolence (66% in our cohort), stereotypic hand movements (36% in our cohort), regression since onset of seizures (38% of individuals with epilepsy in our cohort), soft skin (46% in our cohort), and short stature (19% in our cohort), have not been reported before in published studies. Post-term birth (56% in our cohort), gastro-esophageal reflux disease (28% in our cohort), hypothermia (37% in our cohort), hypotonia after the neonatal period (97% in our cohort), constipation (62% in our cohort), and endocrine abnormalities (47% in our cohort) only have been mentioned in one other study. Similar to many other recently discovered novel syndromes for which only a small subset of individuals have been described initially, frequencies of features related to PURA syndrome were difficult to estimate based on reported numbers. By expanding the cohort, we were able to report a significant number of features not formally associated with PURA syndrome before. These features could be helpful for diagnosis and management of PURA syndrome individuals.

The phenotype of PURA syndrome has been reported as difficult to recognize in daily clinical practice. Here, we show with the identification of one individual using targeted Sanger sequencing in a neonate, that it is possible to recognize PURA syndrome based on clinical features. Severe neonatal problems including hypotonia, respiratory and feeding difficulties, hypersomnolence and hypothermia, in the majority combined with post-term birth, could point towards the early diagnosis of PURA Syndrome. Although not a consistent feature, the presence of myoclonic jerks in a neonate with other features of PURA syndrome may be a useful feature to distinguish from Prader Willi syndrome or peripheral neuromuscular disorders such as SMA. Known syndromes such as Congenital Hypoventilation syndrome[MIM209880], Prader-Willi syndrome[MIM176270], Rett syndrome, Angelman syndrome[MIM160900], Myotonic Dystrophy[MIM160900] and SMA[MIM253300] have been tested often in PURA syndrome individuals. recommend considering PURA syndrome in the differential diagnosis of any child tested for one or more of the above syndromes, especially in the context of the aforementioned neonatal problems and/or unexplained developmental delay.

After diagnosis of PURA syndrome, careful management of existing medical problems and evaluation of health issues associated with PURA syndrome is essential. Our phenotypic overview shows that different organ systems could be affected. Therefore, we recommend routine care in a multidisciplinary team, with at least a (child) neurologist, ophthalmologist, orthopedic surgeon and pediatrician for children aged below 18 years, involved. Epilepsy, present in half of the cohort, is a major problem. Some individuals have never been seizure free and

regression of achieved developmental milestones since onset of seizures has been reported. In future, studies focusing on epilepsy will be important to optimize treatment of seizures related to PURA syndrome.

In general, individuals with epilepsy were more severely affected than individuals without epilepsy. However, this explained only partially the variation in developmental phenotypes observed between individuals. To assess whether the clinical variability could be related to the type and localization of the identified mutations, we performed a genotype-phenotype study by analyzing recurrent mutations and classifying mutations based on a homology model derived from a crystal structure of the *Drosophila* PUR-alpha homologue. Interestingly, remarkable differences, such as the presence or absence of epilepsy or congenital malformations, were observed between individuals with the same mutation. For the remaining mutations, class D mutations with localization at the very end of the C-terminus, could possibly be correlated with a less severe phenotype. But for the other mutations, phenotypic variability and severity could not be related to the localization and type of mutation. Based on these results, we suggest that other genetic and biological mechanisms contribute to the phenotypic variability. Further studies are needed to obtain insight into these underlying pathological mechanisms.

With the identification of 54 individuals within 2.5 years of the initial description of the condition, PURA syndrome appears to be a relatively frequent cause of ID. Previously, frequencies between 0.3% and 0.5% in neurodevelopmental cohorts have been reported.[6-8] Within our cohort, ten individuals were identified in the diagnostic lab facility of the Radboudumc, Nijmegen. These were found in a total of 4700 individuals (~0.2%) with ID referred for WES. This frequency is probably an underestimate. It has been reported that *PURA*, a single-exon gene rich in GC content, could be challenging to capture, similar to the first exon of multiexonic genes.[23] Furthermore, all ten mutations have been identified after the introduction of *PURA* as an ID gene in diagnostic gene panels at the end of 2014, while WES had already been in use in the diagnostic lab of the Radboudumc from 2013. Therefore, to more accurately gauge the frequency within this population, a targeted re-evaluation of all available exome data would be necessary.

In conclusion, we present a detailed overview of the clinical spectrum of PURA syndrome and a computational modeled PURA face, which is essential for better recognition of this newly recognized disorder and surveillance and management of symptoms after initial diagnosis. Our suggested approach for genotype-phenotype analysis by modeling of identified *PURA* mutations into human homology models of experimentally determined crystal structures from *Drosophila* Pur-alpha, showed that clinical variation observed between individuals is probably not related to type and localization of mutations, but should be sought in other genetic and biological mechanisms.

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COMPETING INTERESTS

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FIGURE LEGEND

Figure 1: **Localization of *PURA* mutations and subdivision in classes.** Mutations of individuals identified in our cohort are marked in bold. (A) Homology models of N-terminal and C-terminal PUR domains (grey and blue, respectively) from human Pur-alpha. Residues with single amino acid exchanges are depicted in red with side chains. (B) Location of reported *PURA* frameshift and nonsense mutations. Class A1 mutations are located in the N-terminal PUR domain that affect both N- and C-terminal domains and class A2 mutations occur in the C-terminal domain, affecting only this domain and the C-terminus. (C) Identified point mutations in one of the PUR domains, predicted to cause local folding defects. (D) Four mutations of amino acids located on the protein surface. Three mutations are predicted to affect nucleic-acid binding (Class C) and one mutation (Class D) likely affects a surface-exposed residue, which is not predicted to impair protein folding or nucleic-acid binding. This mutation is possibly involved in not-yet understood functions such as protein-protein interactions. (E) Deletion (red) caused by mutation p.(Met1?) (class E), which disrupts the start codon. The protein is likely expressed from the next in-frame start codon at amino acid 104, causing loss of a functional N-terminal PUR domain, but has an intact C-terminal PUR domain. (F) Localization of mutations reported in healthy controls in the ExAC database. All these mutations are predicted to have no effects on the folding and the function of the Pur-alpha protein.

Figure 2: Photographs of 21 individuals with *PURA* mutations. Shared facial dysmorphism include high anterior hairline, almond-shaped palpebral fissures, full cheeks and hypotonic face. Strabismus is present in several of the PURA individuals. Additionally, independent dysmorphologists also observed eversion of lower lateral eyelids, prominent, well defined philtrum and retrognathia in a subset of the individuals. All legal guardians of the individuals signed written consent for publication of photographs.

Figure 3: Computational analysis of photographs of PURA syndrome individuals.
Result of an objective computational analysis on photographs of 34 individuals with *PURA* mutations (ages at photographs ranging from 2 months to 19 years; right image) compared to the average image based on 301 age-matched, healthy controls (left image). The computational modeled PURA face showed a hypotonic face with typically open mouth appearance and full cheeks. Additionally, two independent dysmorphologists reported a slightly abnormal shape of the eyes as (1) shorter palpebral fissures and (2) eversion of lower lateral eyelids. The high anterior hairline observed in a subset of individuals is not visible on this computational model of the PURA face.

SUPPLEMENTARY DATA

Supplementary Table 1: Detailed information on 32 individuals

Supplementary Table 2: Summarized clinical information on 22 previously published cases

Supplementary Table 3: Results of modeling per mutation

Supplementary Table 4: Phenotypes classified per mutation class

Supplementary Methods: WES in research