Mutations in *PURA* Cause Profound Neonatal Hypotonia, Seizures, and Encephalopathy in 5q31.3 Microdeletion Syndrome

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5q31.3 microdeletion syndrome is characterized by neonatal hypotonia, encephalopathy with or without epilepsy, and severe developmental delay, and the minimal critical deletion interval harbors three genes. We describe 11 individuals with clinical features of 5q31.3 microdeletion syndrome and de novo mutations in *PURA*, encoding transcriptional activator protein Pur-α, within the critical region. These data implicate causative *PURA* mutations responsible for the severe neurological phenotypes observed in this syndrome.

To date, several individuals have been described as having 5q31.3 microdeletion syndrome.[1–4] Shimojima et al.[1] reported two individuals with profound neonatal hypotonia, feeding difficulties, an abnormal electroencephalogram (EEG), hypomyelination, and severe psychomotor delay.[1] Lennox-Gastaut syndrome was diagnosed in one of these individuals, for whom seizures started at 12 months of age. Additional affected individuals were subsequently reported as presenting with marked hypotonia, respiratory insufficiency with central or obstructive sleep apnea, swallowing dysfunction, seizure-like episodes, epilepsy, and neuroimaging abnormalities.[2,3] Although the deletion size varied from 2.6 to 5.0 Mb in the original report,[1] subsequent individuals with smaller deletions narrowed the critical region; the shortest overlapping segment was approximately 101 kb and encompassed purine-rich element binding protein A (*PURA* [MIM 600473; RefSeq accession number NM_005859.4]), IgA-inducing protein (*IGIP*), and cysteine-rich transmembrane module containing 1 (*CYSTM1*).[3] Although *PURA* has earlier been proposed as a candidate gene with mutations responsible for the neurological phenotypes in this syndrome,[3] no individuals with point mutations in this gene have been described.

We identified 11 individuals with de novo heterozygous mutations in the single-exon gene *PURA* among 2,908 consecutive subjects referred to the Whole Genome Laboratory at Baylor College of Medicine from October 2011 to April 2014 for clinical exome sequencing.[5] Approximately 70% of the individuals were pediatric subjects with a variable spectrum of neurodevelopmental disorders. Sequencing and data analysis were conducted as previously described (Yang et al.[5]) and targeted ~20,000 genes, including the coding and UTR exons. Average coverage for targeted regions was greater than 130×, and more than 95% of the target bases were covered by at least 20 reads. To clarify the role of *PURA* in 5q31.3 microdeletion syndrome, we analyzed previously unreported *PURA* variants found by clinical exome sequencing. Subjects were enrolled in research studies approved by the institutional review board of Baylor College of Medicine or the University of Rochester, and informed consent was obtained. Sanger sequencing confirmed the *PURA* mutations in all individuals and validated the absence of the relevant mutations in both parents for each of the 11 probands. For each proband, samples were obtained from the biological parents, and the transmission of multiple rare variants was verified by Sanger sequencing confirming the stated

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parental relationship. Of the identified *PURA* mutations, four were truncating (three nonsense and one frameshift), five were missense, and two were in-frame deletions (Table 1; Figure 1A). None of these mutations were detected in 1000 Genomes (release 20110521), dbSNP134, the NHLBI Exome Sequencing Project Exome Variant Server, or the database of Atherosclerosis Risk in Communities (exome data of ~6,000 subjects). No additional contributing mutations in other genes were identified in the 11 individuals with de novo *PURA* variants.

The phenotype of these 11 probands (Table 1; Table S1, available online) is distinctly similar to that of 5q31 microdeletions encompassing this gene.[1–3] All 11 individuals presented at birth with significant hypotonia. Respiratory insufficiency, including central and obstructive sleep apnea and recurrent pulmonary aspiration, were frequently observed. Early-onset feeding difficulties with moderate dysphagia and evidence of tracheal aspiration often necessitated nasogastric or gastric-tube feeding. Myopathic facies with an open mouth and high arched palate were common features (Figure 1B), as observed in the subjects with 5q31.3 microdeletion syndrome.[1–3] Hypomyelination or myelin-maturation delay was noted on brain-imaging studies in 4/11 individuals. Myoclonic jerks in infancy were frequent, leading 6/11 children to progress to develop epilepsy. An abnormal EEG was documented in most individuals. Lennox-Gastaut syndrome was diagnosed in two individuals. Almost all of the affected children were nonverbal and nonambulatory at the time of evaluation. Prior to exome sequencing, many of these individuals had undergone extensive diagnostic evaluations, including DNA sequencing and biochemical studies for genetic conditions, such as Prader-Willi and Angelman syndromes, spinal muscular atrophy, congenital disorders of glycosylation, peroxisomal disorders, Rett syndrome, and mitochondrial disease. Our results suggest that individuals with *PURA* mutations share a recurring pattern of clinical features composing a recognizable phenotype and potentially establish a link between the severe neurodevelopmental profile of 5q31.3 microdeletion syndrome and transcriptional activator protein Pur-α.[6]

PURA encodes a highly conserved 322 amino acid multifunctional protein, Pur-α, that has important roles in DNA replication, DNA transcription, and mRNA trafficking.[7] Pur-α is required for postnatal development of the murine brain and is involved in both neuronal proliferation and maturation of dendrites.[8,9] It has also been shown to be important for the transport of specific mRNA molecules to sites of translation in hippocampal neurons.[10] Homozygous *Pura*$^{-/-}$ mice appear normal at birth, but they develop tremors and seizures at 2 weeks and die by 4 weeks of age.[8] The number of neurons is severely lower in the cerebellum, cortex, and hippocampus of these mice than in those of age-matched *Pura*$^{+/+}$ controls.[8] The development of dendrites is also abnormal in the cerebellum and hippocampus of *Pura*$^{-/-}$ mice.[10] Pur-α has been found to colocalize with fragile X mental retardation protein 1 homolog (FMRP) at several sites throughout dendrites in mice.[10] *Pur-α*, the *Drosophila* ortholog of *PURA*, has been shown to interact with the RNA transcript of *FMR1* at the rCGG repeats and has been implicated in neurodegeneration in the model of fragile-X-associated tremor/ataxia syndrome.[11] It is postulated that this interaction might be necessary for *FMR1* mRNA transport into dendrites. Similarly, Pur-α has been shown to be involved in the transport of mRNAs into the growing oocytes in *Drosophila*, given its expression in both follicle cells and germline cells.[12] The crystal structure of the protein reveals that the functionality of Pur-α relies on the three conserved PUR motifs.[13] The first and second PUR motifs function in binding of single-stranded DNA or RNA, whereas the third PUR motif is involved in the dimerization of Pur-α. In previous studies, targeted mutations in critical codons within the PUR motifs abolished the ability of Pur-α to bind DNA or RNA and impaired the translocation of Pur-α from follicle or nurse cells into oocytes.[12,13] The mutation in codon 97 of subject 4 overlaps the *Drosophila* critical codon 80 mutation (causing p.Arg80Ala), previously[12,13] shown to result in reduced nucleic acid binding without affecting correct folding. The missense and in-frame-deletion mutations in our study are all located at conserved sites within PUR motifs (Figure S1). Two in silico prediction tools, PolyPhen-2 and SIFT, predict that the five missense mutations have damaging effects (Table S2). Interestingly, given that this is a single-exon gene and thus not likely subject to nonsense-mediated decay, all truncating mutations found in our series will result in loss or partial loss of PUR motif(s).

To further elucidate the biological roles of *PURA*, we employed the model organism *Caenorhabditis elegans*, whose *PURA* ortholog is *plp-1*. The *plp-1*-null allele, *ok2155*, has a 1,107 bp deletion encompassing part of the promoter region and the first 255 bp of cDNA sequence involving two exons (Figure S2). We found that homozygous *plp-1* (*ok2155*) mutants were sterile. DAPI staining showed that oocytes were absent in the *plp-1* mutant (Figures 1C and 1D), suggesting a requirement of *plp-1* for germline differentiation. The *plp-1* mutants had defective locomotion, marked by a 3-fold reduction in speed in comparison to wild-type animals (Figure 1E; Movies S1 and S2). Compared to age-matched wild-type worms, which showed normal movement, the mutants exhibited minimal forward movement across the culture medium. The observed defects might be related to the impaired mRNA trafficking during oogenesis and neuronal activities. Together, these results suggest the essential functions of *plp-1* in both somatic and germline tissues. Human *PURA* is expressed in the brain and ovarian tissues (Figure S3) and might have roles similar to those of *plp-1*.

In conclusion, we characterize the clinical features and report de novo heterozygous *PURA* mutations in multiple individuals with a significant neurodevelopmental phenotype recapitulating 5q31.3 microdeletion

Table 1. Clinical Features of Individuals with *PURA* Mutations

	Subject											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Summary**
Gender	male	male	male	female	female	female	female	female	male	female	female	four males, seven females
Age at evaluation	6 months	7 months	10 months	21 months	23 months	2 years	2 years	5 years	12 years	12 years	15 years	NA
Mutation	c.812_814delTCT (p.Phe271 del)	c.307_308delTC (p.Ser103Hisfs*97)	c.556C>T (p.Gln186*)	c.289A>G (p.Lys97Glu)	c.299T>C (p.Leu100Pro)	c.363C>G (p.Tyr121*)	c.783C>G (p.Tyr261*)	c.470T>A (p.Met157Lys)	c.265G>C (p.Ala89Pro)	c.263_265delTCG (p.Ile88_Ala89delinsThr)	c.596G>C (p.Arg199Pro)	four truncating, seven nontruncating
Inheritance	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	11/11
Hypotonia	+	+	+	+	+	+	+	+	+	+	+	11/11
Feeding difficulties	+	+	+	+	+	+	+	+	+	+	+	11/11
Respiratory difficulties	+	+	+	+	+	+	+	+	+	+	ND	10/10
Seizures	+	–	+	+	+	+	+	–	+	+	+	9/11
Seizure onset	3 weeks	–	neonatal	ND	5 months	3 months	neonatal	–	3 years	4 years	ND	NA
Seizure type	myoclonic, secondary generalized tonic	–	myoclonic jerks	myoclonic jerks, exaggerated startle	generalized tonic clonic	myoclonic jerks	myoclonic jerks	–	myoclonic, generalized tonic and atonic	Lennox-Gastault syndrome	Lennox-Gastault syndrome	NA
Abnormal EEG	+	–	+	ND	ND	+	+	ND	+	+	+	NA
Psychomotor delay	+	+	+	+	+	+	+	+	+	+	+	11/11
Nonverbal	ND	ND	ND	+	+	+	+	+	+	+	+	8/8
Ambulatory	ND	ND	ND	–	–	–	–	–	–	walked at 3.5 years, ataxia	–	1/8
Craniofacial features	nondysmorphic	high arched palate, upturned nose, simple lobulation of ears, intermittent exotrophia	nondysmorphic	myopathic facies, strabismus	myopathic facies, tented upper lip, nystagmus	myopathic facies	myopathic facies, left-eye esotropia	nondysmorphic, nystagmus, strabismus	myopathic facies, high arched palate, nystagmus	myopathic facies, high arched palate, wide nasal bridge and tip, hypoplastic alae nasi, strabismus, myopia	nondysmorphic, palpebral fissures long, nystagmus	NA

See Table S1 for additional details of clinical presentations. Abbreviations are as follows: EEG, electroencephalogram; NA, not applicable; and ND, not determined because of availability or applicability.

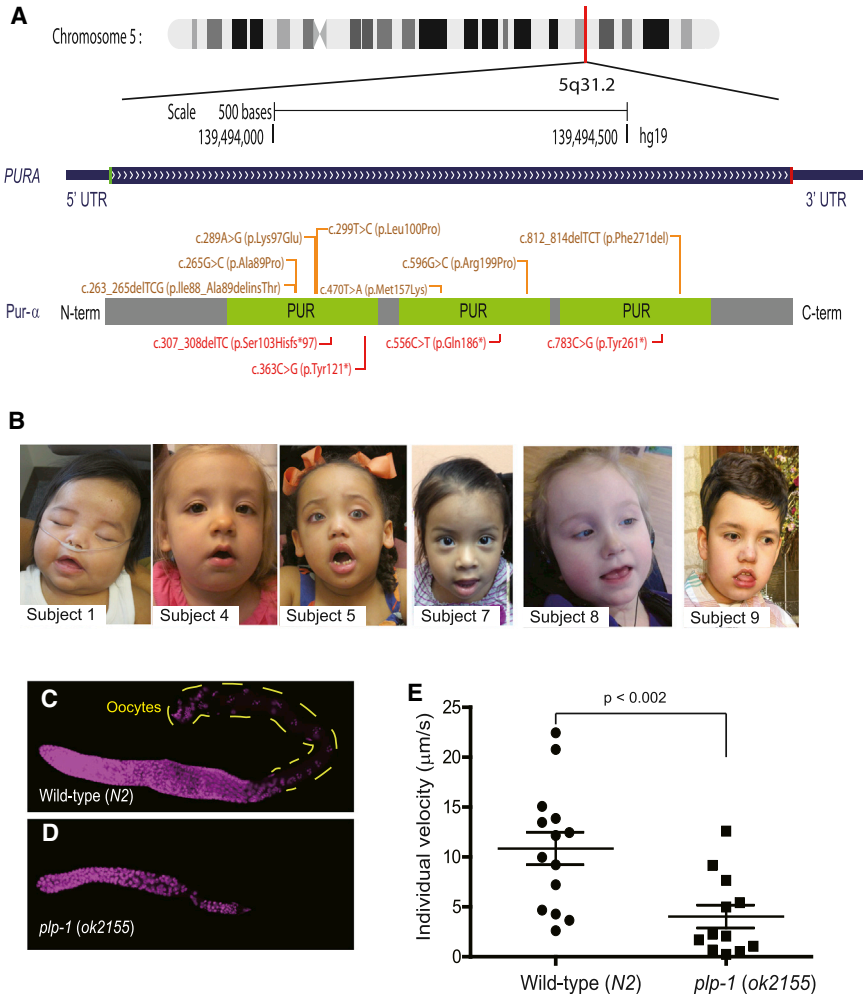

Figure 1. *PURA* Mutations in the Affected Human Subjects and Functions of the *PURA* Ortholog in Model System *C. elegans*

(A) *PURA* is located in chromosomal region 5q31.2 (according to the UCSC Genome Browser). A single exon (blue) encodes a protein with three PUR motifs, as shown in green. On the protein diagram, missense and in-frame-deletion mutations are brown, and the truncating mutations are shown in red.

(B) Six subjects with de novo *PURA* mutations are shown. The craniofacial features are mainly characterized by myopathic facies. For whom clinical photographs are shown, informed consent specifically agreeing to publish these photographs in medical publications was obtained.

(C) DNA staining in the germline of a wild-type *C. elegans* animal. Yellow dashed lines indicate differentiated oocytes.

(D) Oocytes were absent in the *plp-1*-mutant animal with smaller gonads. Wild-type (N2) and RB1711 (*plp-1(ok2155)IV*) animals were obtained from the Caenorhabditis Genetics Center. *C. elegans* were grown on standard nematode growth media plates (containing 5 mg/l cholesterol) with *E. coli* OP50 at 25°C according to standard protocols. DAPI staining was performed at room temperature. The germline was dissected out from young adult worms.

(E) Average speed of individual worm movement (wild-type n = 14, *plp-1* n = 12). The p value was obtained by a one-tailed Student's t test. Error bars in the scattered dot plot represent the mean and the SEM. Spontaneous movement of age-synchronized worms on standard worm plates was recorded for 15 s with an SMZ1500 stereo microscope (Nikon) connected to a C11440 camera (Hamamatsu). Individual worms were tracked with NIS Elements AR 2D tracking imaging software (Nikon), and the average velocity (μm/s) was calculated. Representative recording of wild-type worms and *plp-1* mutants are shown in Movies S1 and S2, respectively.

syndrome. Considering all 2,117 pediatric subjects who had a neurodevelopmental disorder and were studied by clinical exome sequencing in our laboratory, the 11 heterozygous mutations in *PURA* account for ~0.52% and are exceeded only by those in well-established genes AT-rich interactive domain 1B (SWI1-like) (*ARID1B* [MIM 614556]; 21 subjects [0.99%]) and ankyrin repeat domain 11 (*ANKRD11* [MIM 611192]; 13 subjects [0.61%]). Notably, our observed frequency of pathogenic events in *ARID1B* is consistent with the 0.9% rate previously reported by Hoyer et al.[14] *PURA* mutations should be considered in the evaluation of infants with profound neonatal hypotonia, myoclonic jerks, respiratory insufficiency, and an abnormal EEG. Although further investigations are essential for understanding the effects of *PURA* haploinsufficiency on encephalopathy with or without epilepsy, this study is an important step toward the clinical and molecular characterization of this disease, which is typically associated with a significant neurological outcome.

Supplemental Data

Supplemental Data include three figures, two tables, and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2014.09.014.

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Web Resources

The URLs for data presented herein are as follows:

1000 Genomes, http://www.1000genomes.org/
Atherosclerosis Risk in Communities Study, http://www2.cscc.unc.edu/aric/
Baylor College of Medicine Whole Genome Laboratory, https://www.bcm.edu/research/medical-genetics-labs/wholegenomelab
ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/
NHLBI Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/
PolyPhen2, http://genetics.bwh.harvard.edu/pph2/
RefSeq, http://www.ncbi.nlm.nih.gov/refseq/
SIFT, http://sift.jcvi.org/

Accession Numbers

The ClinVar accession numbers for the DNA variant data reported in this paper are SCV000189113, SCV000189114, SCV000189115, SCV000189116, SCV000189117, SCV000189118, SCV000189119, SCV000189120, SCV000189121, SCV000189122, and SCV000189123.

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Supplemental Data

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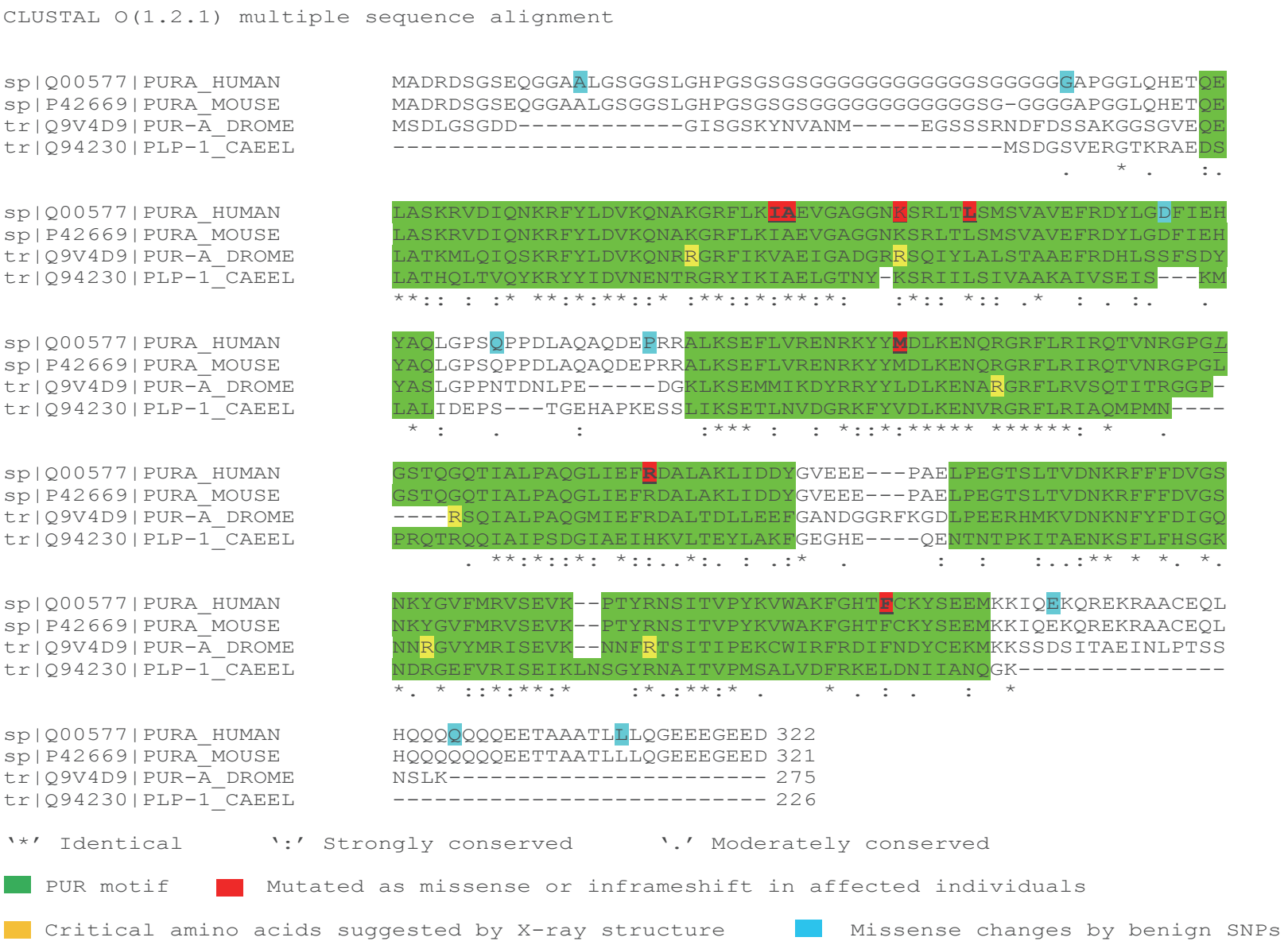


Figure S1. Sequence alignment of PURA family proteins in human (Q00577), mouse (P42669), *Drosophila* (Q9V4D9) and *C. elegans* (Q94230). The sequences were from the UniProt database (http://www.uniprot.org/).

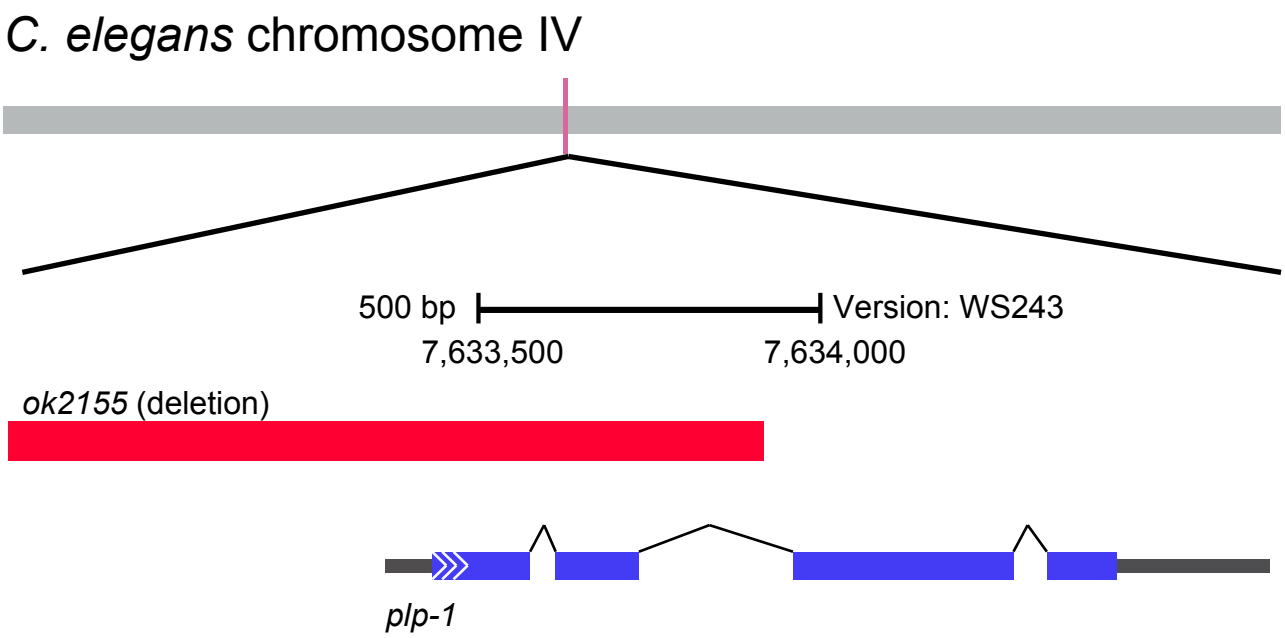
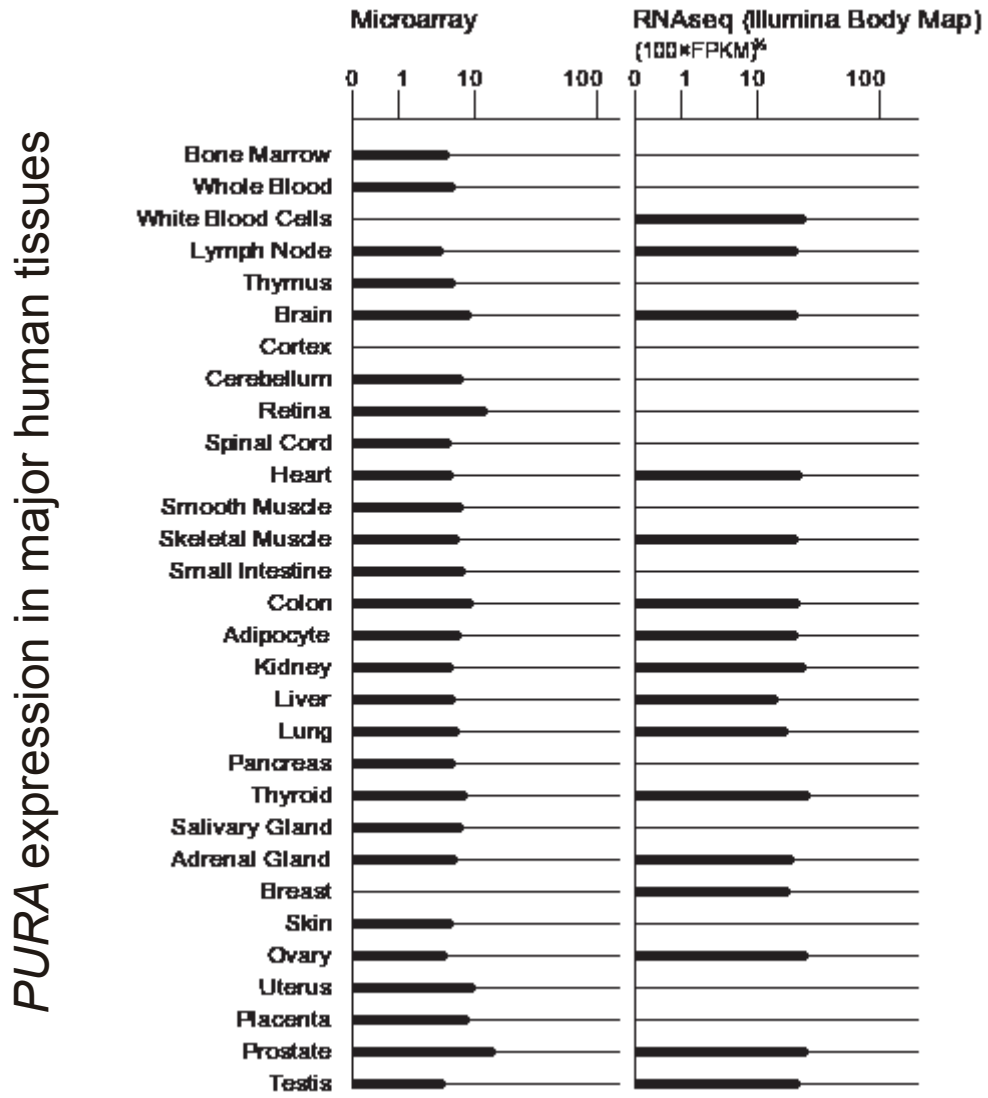


Figure S2. Genomic structure of the ***plp-1*** gene in ***C. elegans*** and the ***ok2155*** deletion allele (red). Derived from Wormbase (http://www.wormbase.org/).



From http://www.genecards.org/

Figure S3. Expression pattern of *PURA* indicated by public microarray and RNA-seq data. The bars show the relative levels of *PURA* mRNA in major tissues. Derived from GeneCards® (http://www.genecards.org/).

Table S2. *In silico* predictions of missense *PURA* mutations

Gene	RefSeq ID	cDNA	Protein	SIFT Score	SIFT Effect	PolyPhen2 Score	PolyPhen2 Effect
PURA	NM_005859.4	c.265G>C	p.Ala89Pro	0	Damaging	1	Probably damaging
PURA	NM_005859.4	c.289A>G	p.Lys97Glu	0	Damaging	1	Probably damaging
PURA	NM_005859.4	c.299T>C	p.Leu100Pro	0	Damaging	1	Probably damaging
PURA	NM_005859.4	c.470T>A	p.Met157Lys	0	Damaging	0.954	Possibly damaging
PURA	NM_005859.4	c.596G>C	p.Arg199Pro	0	Damaging	1	Probably damaging