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Supplemental Data

Biallelic Mutations in *UNC80* Cause Persistent Hypotonia, Encephalopathy, Growth Retardation, and Severe Intellectual Disability

Asbjørg Stray-Pedersen, Jan-Maarten Cobben, Trine E. Prescott, Sora Lee, Chunlei Cang, Kimberly Aranda, Sohnee Ahmed, Marielle Alders , Thorsten Gerstner, Kathinka Aslaksen, Martine Tétreault, Wen Qin, Taila Hartley, Shalini N. Jhangiani, Donna M. Muzny, Maja Tarailo-Graovac, Clara D.M. van Karnebeek, Care4Rare Canada Consortium, Baylor-Hopkins Center for Mendelian Genomics, James R. Lupski, Dejian Ren, and Grace Yoon

Supplemental Data

Supplemental Case Histories and Figures

Subject F1-IV.1

This is a four-year-old girl who is the only child of healthy Iraqi parents who are first cousins. Family history is otherwise non-contributory. She was born via spontaneous vaginal delivery at 41 weeks gestation after a normal pregnancy, with a birth weight of 3000 g and Apgar scores of 9 at one and five minutes**.** She was hypotonic, with feeding difficulties and severe constipation. At age 2 weeks, she was investigated for possible Hirschsprung's disease; however, a rectal biopsy was normal. At age three months she developed generalized tonic-clonic seizures. These were well-controlled with Clonazepam, and she has been seizure-free for the past year. A subglottic web was detected in conjunction with investigations for frequent recurrent febrile episodes between ages four and nine months. At age 2 years a G-tube was placed due to severe feeding difficulties, however she continues to fail to thrive. Currently at age four years, she is able to sit independently for a few seconds. She rolled from supine to prone at age three years, but is not able to crawl, stand or walk, and does not have a pincer grasp. She can bring her hands to the midline and transfer objects from hand to hand. She babbles but does not have any speech, and does not follow commands. She has little interest in her surroundings, and makes occasional eye-contact. She has severe sleep disturbance with reversed sleep-wake cycle, despite treatment with melatonin 4 mg qhs, and obstructive sleep apnea. She also has choreiform movements, hypothyroidism and superficial punctate keratopathy.

On examination at age four years, her height was 72 cm (well below the 3rd centile, 50th centile for 10 months), weight 7.4 kg (well below the 3rd centile, 50th centile for 6 months), and occipito-frontal head circumference (OFC) 43 cm (-4 standard deviations, well below the 3rd centile). She was profoundly hypotonic and brachycephalic with generalized joint laxity. Craniofacial features included bilateral epicanthal folds, bulbous nasal tip, thin vermillion border of the upper lip and low-set ears. (Figure 1A) She was able to make anti-gravity movements of all four limbs but not against resistance. Deep tendon reflexes were 2+ and plantar responses were flexor. Sensation appeared normal. She had occasional choreiform movements but no tremor.

The following investigations were reported as normal: visual evoked potentials, magnetic resonance spectroscopy of the brain, chromosomal microarray (4×180K Cytosure ISCA v2 oligonucleotide platform, Oxford Gene Technology Inc., Oxford, UK), chromosomal breakage studies in blood lymphocytes, plasma amino acids, urine organic acids, plasma lactate, plasma carnitine, plasma thymidine, urinary glycosaminoglycans and oligosaccharides, cerebrospinal fluid analyses (protein, glucose, amino acids, lactate and neurotransmitter metabolites), as well as transferrin iso-electric focusing studies.
An EEG was suggestive of encephalopathy at age 10 months. MRI of the brain revealed global reduction in cerebral volume, a thin corpus callosum, and myelination at the lower limit of normal. Mild non-specific myopathic changes were evident on muscle biopsy.

Subject F2-V.5

This is a 3.9-year-old girl born at 41 weeks after a normal pregnancy. The healthy parents, who are originally from Morocco, are first cousins once removed and she is their fifth child. Family history is otherwise non-contributory. Birth weight was 3158 g and Apgar scores were 8/10/10 after 1/5/10 minutes respectively. Hypotonia was noted from birth but was more prominent at age three months. At age 3.9 years she is a small child with generalized hypotonia. She has been able to sit without support since age 2.5 years but cannot stand. She makes sounds, but has no speech. She has myopathic facies, ptosis, and shallow nasolabial folds. (Figure 1B) Onset of epilepsy with tonic-clonic seizures was at age three years and 10 months. A G-tube was inserted at 3 years for feeding problems and recurrent upper airway infections thought to be related to malnourishment. The following investigations were reported as normal: metabolic screening in urine and blood (age 10 months), chromosomal microarray (Agilent 180K Oligoarray), methylation analysis of the Silver-Russell syndrome critical regions on chromosomes 11 and 7, brain MRI (age 10 months), EEG (age 10 months), opthalmologic exam, visual evoked potentials (age two years and 11 months), brainstem auditory evoked potentials (age 2 years 11 months).

Subject F3-II.1 and Subject F3-II.3

These sisters were born to non-consanguineous healthy parents of Norwegian ethnicity at term with normal birth weights after uncomplicated pregnancies. At ages 15 and 9 years respectively, they remain extremely hypotonic and do not walk without support. Both are normocephalic with OFC in the low normal range. They babble socially, have very communicative body language but no meaningful speech. They are generally content

children but sudden sounds, movements or changes in ambient light frighten them. Tactile aversion, particularly on the soles of the feet, was present in both until about age two years. Chronic constipation has required medical intervention in both. They do not have involuntary movements or stereotypic hand movements and sleep well. They have been treated conservatively for esotropia and the younger girl uses glasses. Vision and hearing appear otherwise normal. On exam, they are short thin girls with dark circles infraorbitally, eyelid ptosis, alternating esotropia, hypotonic facies (Figures 1 C, D) and small hands and feet.

Subject F3-II.1, who was more hypotonic at birth than her sister, was an undemanding baby who slept a lot. She now pulls to a stand from sitting, walks with a walker for up to an hour at a time and uses her hands to bring food to her mouth. Epilepsy was diagnosed at age three years, but in retrospect she most likely had atonic seizures from age six months. Her seizure disorder has been difficult to manage from around age 10 years and a vagal nerve stimulator was implanted at age 12 years. She has only recently had a G-tube placed in order to deliver anti-convulsants more reliably and to supplement oral caloric intake when needed. Her daytime sleepiness seems related to seizure activity and side effects of medications. She has developed a mild scoliosis.

Subject F3-II.3 has been completely G-tube fed since age 11 months. She reacts to nutritional supplements with malaise and vomiting, and therefore receives the majority of her caloric intake as liquefied regular food. She has gastro-oesophageal reflux that

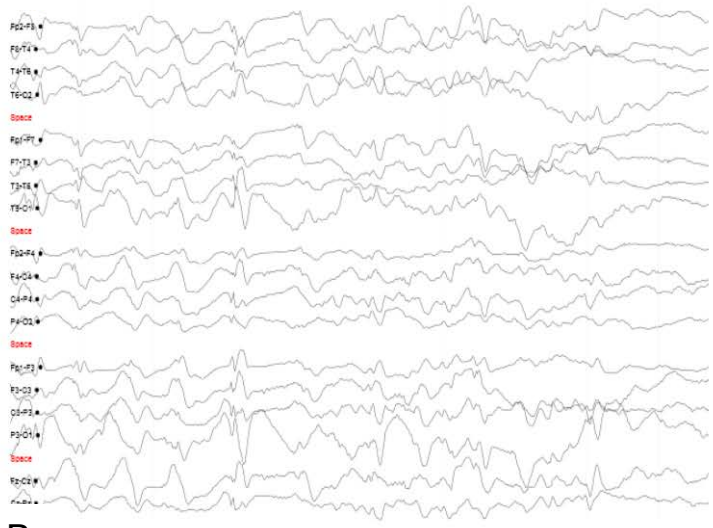
responds to medical treatment. She can manoeuvre from the prone to sitting without assistance and sits stably while using both arms to manipulate toys.

The sisters have experienced multiple seizure types (Table 1), but both display a strikingly similar EEG pattern characterized by encephalopathic background activity with frequent spike and wave discharges (Figure S1).

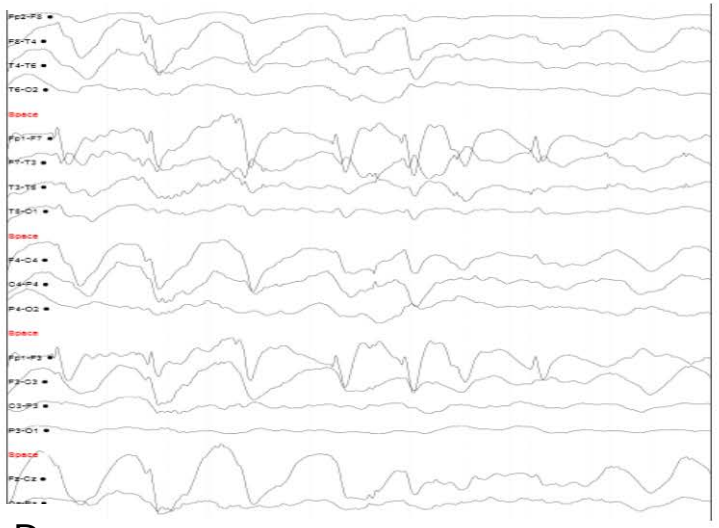
Cranial MRI was normal in both as were the following investigations: expanded metabolic screening in blood and urine, molecular analysis of *UBE3A, MECP2, MEF2C, FOXG1, CDKL5, TCF4, CNTNAP2, NTNG1, SLC6A8, ZFHX1B/ZEB2, NRXN1, SCN1A, MTHFR* and *DMPK*. Chromosomal microarray with Agilent 105K oligoarray and 250K SNP array showed no disease causing CNVs or regions with absence of heterozygosity.

Supplemental Figures

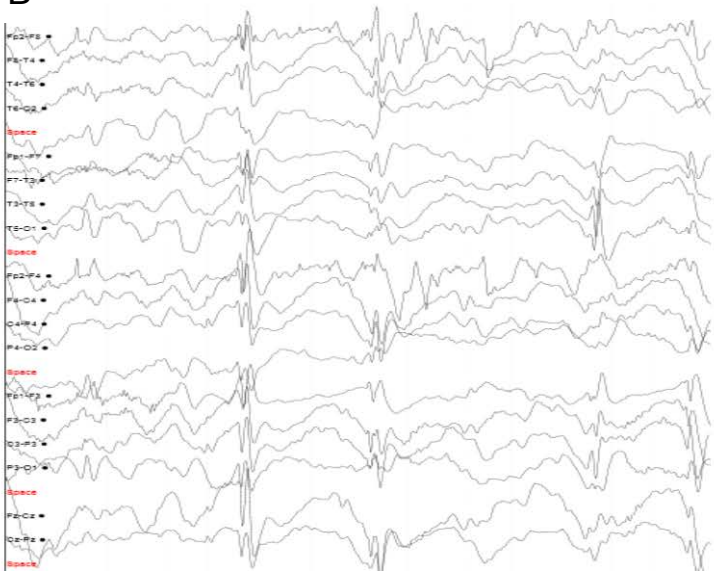
A



C



B



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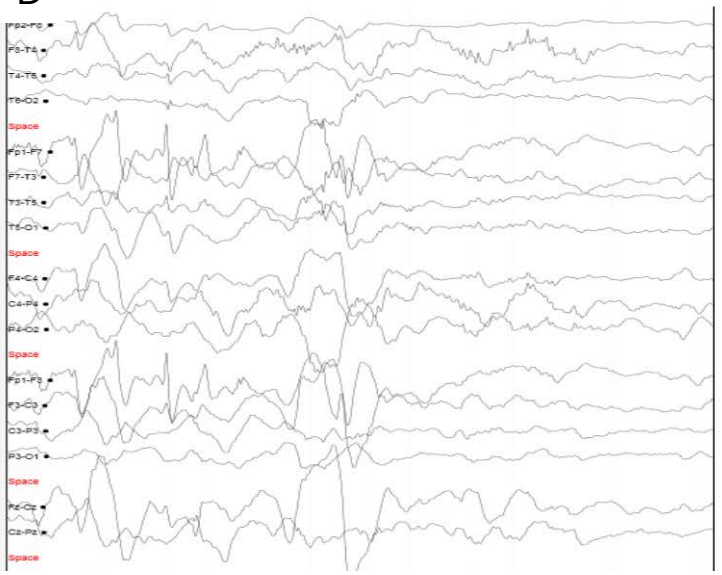

Figure S1. Electroencephalograms (EEGs) from Family 3. EEG of F3-II.1 at 14.5 years of age (**A**) during sleep (**B**) awake. EEG of F3-II.3 at 9 years of age (**C**) during sleep (**D**) awake. The EEGs shows an encephalopathic background with generalized delta-theta activity 3-6 Hz and periodic sharp-slow waves and sharp waves with a fronto-central or multifocal maximum. While sleeping the background activity slowed down to 2-3 Hz with discharges of high amplitude sharp slow waves with a frontal maximum. This EEG pattern observed in the sisters is reminiscent of the pattern seen in individuals with Angelman syndrome. Calibration: longitudinal bipolar montage, 1 second per horizontal unit, 50 μV per vertical unit. Low cut filter 0,53 sec, high cut filter 70Hz, sensitivity 15 μV/mm, Notch on.

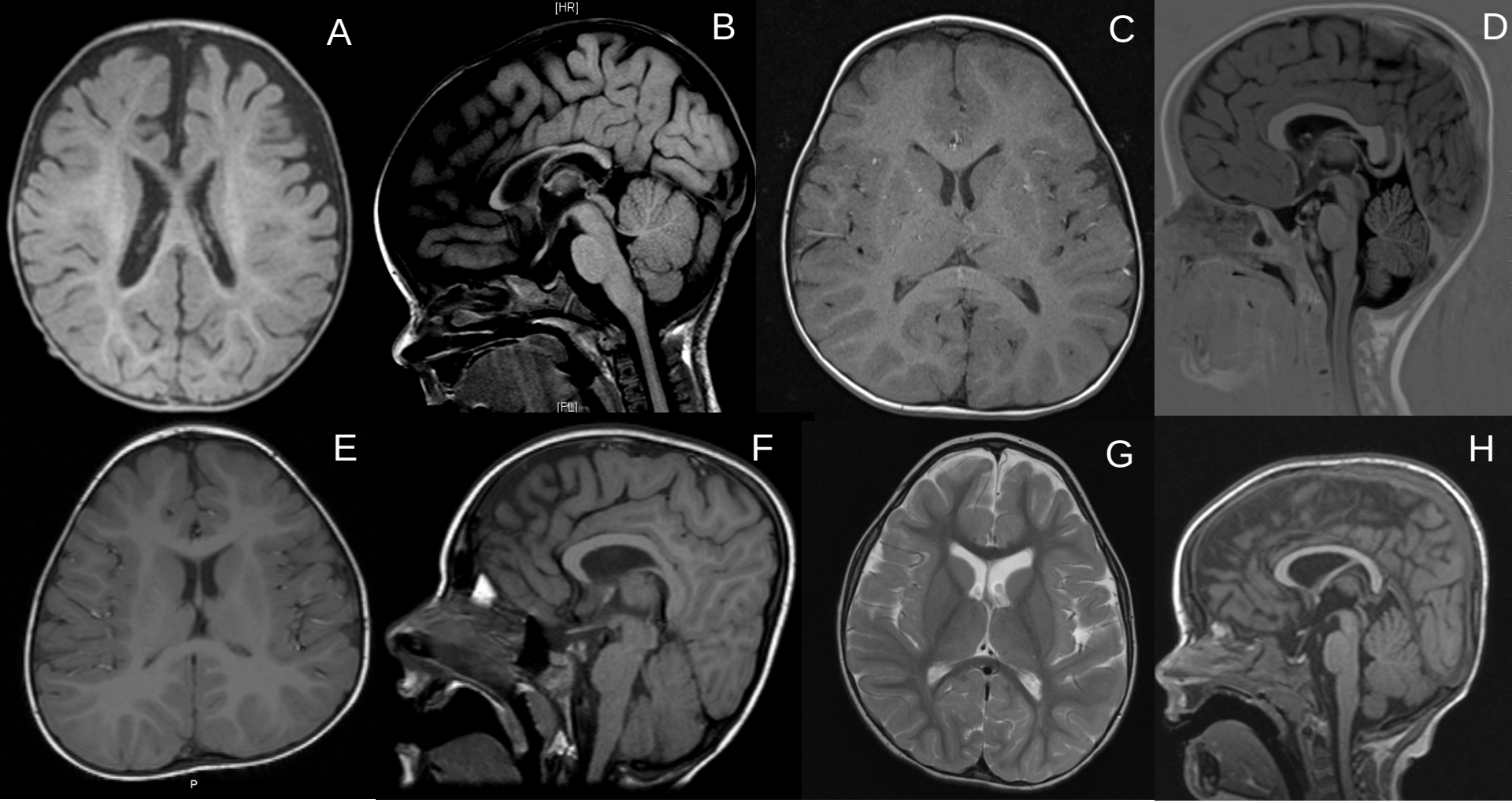
A
B
C
D
E
F
G
H

Figure S2. Neuroimaging results. (A) Axial T1-weighted image and (**B**) sagittal T1-weighted image of Subject F1-IV.1 at 10 months. There is global reduction of cerebral volume and thinning of the corpus callosum. (**C**) Axial T1-weighted image and (**D**) sagittal T1-weighted image of F2-V.5 at 2 years, (**E**) Axial T1-weighted image and (**F**) sagittal T1-weighted image of F3-II.1 at 6 years, (**G**) Axial T1-weighted image and (**H**) sagittal T1-weighted image of F3-II.2 at 2.5 years, demonstrating normal results.

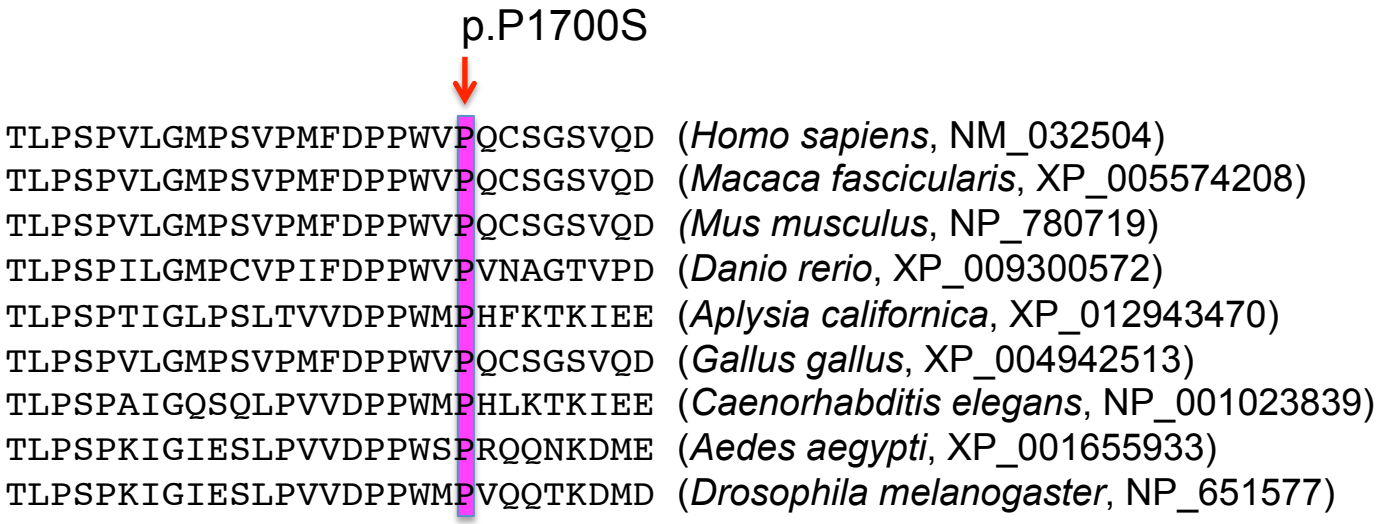
p.P1700S
TLPSPVLGMPSVPMFDPPWVPQCSGSVQD (*Homo sapiens*, NM_032504)
TLPSPVLGMPSVPMFDPPWVPQCSGSVQD (*Macaca fascicularis*, XP_005574208)
TLPSPVLGMPSVPMFDPPWVPQCSGSVQD (*Mus musculus*, NP_780719)
TLPSPILGMPCVPIFDPPWVPVNAGTVPD (*Danio rerio*, XP_009300572)
TLPSPTIGLPSLTVVDPPWMPHFKTKIEE (*Aplysia californica*, XP_012943470)
TLPSPVLGMPSVPMFDPPWVPQCSGSVQD (*Gallus gallus*, XP_004942513)
TLPSPAIGQSQLPVVDPPWMPHLKTKIEE (*Caenorhabditis elegans*, NP_001023839)
TLPSPKIGIESLPVVDPPWSPRQQNKDME (*Aedes aegypti*, XP_001655933)
TLPSPKIGIESLPVVDPPWMPVQQTKDMD (*Drosophila melanogaster*, NP_651577)

Figure S3. Alignment of UNC80 sequences in the Pro1700 region. Species and GenBank accession numbers are in parentheses. Arrow indicates the p.Pro1700Ser variant in Subject F1-IV.1.


NM_032504.1(UNC80):c.7607G>C - [c.7505 (Exon 50) - c.7607+97 (Intron 50)]
Alamut Visual v.2.7 rev. 1
SpliceSiteFinder-like
MaxEntScan
NNSPLICE
GeneSplicer
Human Splicing Finder
5'
3'
Reference Sequence
Mutated Sequence
Branch Points
[0-100]
[0-12]
[0-1]
[0-15]
[0-16]
68.7
7.7
0.7
6.1
75.2
0.0
1.9
79.0
55.4
0.1
64.2
2.2
7580
7590
7607
7607+10
7607+20
7607+30
AACTGCTGGATGTGAAGTCTCACATGAGGTACTGGCCTCGCTTTCCCTGCCCCAAGTGTGAACA
AACTGCTGGATGTGAAGTCTCACATGACGTACTGGCCTCGCTTTCCCTGCCCCAAGTGTGAACA
interactive
biosoftware

Figure S4. Predicted disruption of splicing for the c.7607G>C variant found in Subject F2-V.5. Potential consequences of the c.7607G>C variant on splicing were analyzed using five prediction methods (SpliceSiteFinder-like, MaxEndScan, NNSPLICE, GeneSplicer and Human Splicing Finder; performed with Alamut Visual of Interactive Biosoftware). Ranges of score are indicated in the parentheses right to the methods. Blue and green vertical bars indicate predicted 5' donor and 3' acceptor sites, respectively, with scores indicated for those varying between the wild-type and the variant. Sites with scores unchanged between the wild-type and the variant are dimmed.

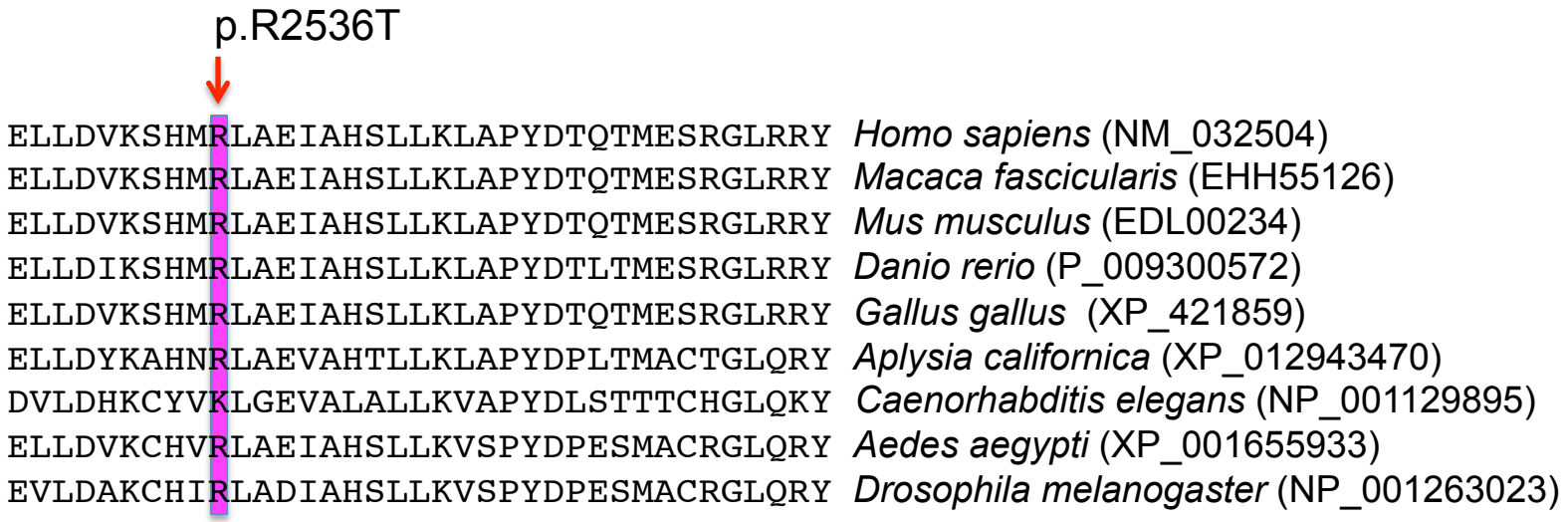
p.R2536T
ELLDVKSHMRLAEIAHSLLKLAPYDTQTMESRGLRRY Homo sapiens (NM_032504)
ELLDVKSHMRLAEIAHSLLKLAPYDTQTMESRGLRRY Macaca fascicularis (EHH55126)
ELLDVKSHMRLAEIAHSLLKLAPYDTQTMESRGLRRY Mus musculus (EDL00234)
ELLDIKSHMRLAEIAHSLLKLAPYDTLTMESRGLRRY Danio rerio (P_009300572)
ELLDVKSHMRLAEIAHSLLKLAPYDTQTMESRGLRRY Gallus gallus (XP_421859)
ELLDYKAHNRLAEVAHTLLKLAPYDPLTMACTGLQRY Aplysia californica (XP_012943470)
DVLDHKCYVKLGEVALALLKVAPYDLSTTTCHGLQKY Caenorhabditis elegans (NP_001129895)
ELLDVKCHVRLAEIAHSLLKVSPYDPESMACRGLQRY Aedes aegypti (XP_001655933)
EVLDAKCHIRLADIAHSLLKVSPYDPESMACRGLQRY Drosophila melanogaster (NP_001263023)

Figure S5. Alignment of UNC80 sequences in the Arg2536 region. Species and GenBank accession numbers are in parentheses. Arrow indicates the p.Arg2536Thr variant in Subject F2-IV.5.

Table S1. Summary of key clinical features in individuals with biallelic mutations in *UNC80* or *NALCN*

	Individuals with *UNC80* mutations				**Individuals with *NALCN* mutations**							
Publication	This report				Köroğlu Ç et al, 2013		Al-Sayed MD et al, 2013					
	Family 1	Family 2	Family 3		Family 5		Family 1			Family 2		
Age (years)	4	4	15	9	20+	20+	7	7	4	17	16	9
Gender	Female	Female	Female	Female	Female	Male	Male	Male	Male	Female	Female	Female
Age at presentation	Birth	Birth	Birth	3 months	Birth	Birth	Birth	Birth	Birth	Birth	Birth	Birth
Persistent hypotonia	+	+	+	+	+	+	+	+	+	-	-	-
Growth history	Failure to thrive, G-tube dependent	Failure to thrive, G-tube dependent	Failure to thrive, supplemental G-tube use	G-tube dependent	Failure to thrive	Failure to thrive	normal	Initial failure to thrive followed by normal growth	Initial failure to thrive followed by normal growth	normal	normal	normal
Severe Intellectual disabilit	+	+	+	+	+	+	+	+	+	+	+	+

y												
Communication	Non-verbal	Non-verbal	Non-verbal	Non-verbal	Non-verbal	Non-verbal	Non-verbal	Non-verbal	Non-verbal	2-word phrases	2-word phrases	2-word phrases
Gross motor function	Non-ambulatory	Non-ambulatory	Walks with support	Walks with support	Non-ambulatory	Non-ambulatory	Non-ambulatory	Non-ambulatory	Non-ambulatory	Walks independently	Walks independently	Walks independently
Seizures	+	+	+	+	+	+	-	-	-	+	+	+
Constipation	+	+	+	+	+	+	+	+	+	+	+	+
Dysmorphic facial features	Hypotonic facies	Hypotonic facies	Hypotonic facies	Hypotonic facies	Prominent forehead, low-set ears, small nose, micrognathia	Prominent forehead, low-set ears, small nose, micrognathia	Triangular facies, prominent nose, smooth philtrum	Triangular facies, prominent nose, smooth philtrum	Triangular facies, prominent nose, smooth philtrum	Triangular facies, prominent nose, smooth philtrum	Triangular facies, prominent nose, smooth philtrum	Triangular facies, prominent nose, smooth philtrum
Brain MRI	Global reduction in cerebral volume, thin corpus callosum	Normal	Normal	Normal	Normal	Cerebellar atrophy	normal	Bilateral parietal white matter abnormalities	Small posterior fossa	normal	normal	normal
Mutation (s)	c.5098 C>T (homozygous)	c.7607 G>C (homozygous)	c.7757T>A/c.2033delA	c.7757T>A/c.2033delA	c.1924 C>T (homozygous)	c.1924 C>T (homozygous)	c.1489delT (homozygous)	c.1489delT (homozygous)	c.1489delT (homozygous)	c.3860 G>T (homozygous)	c.3860 G>T (homozygous)	c.3860 G>T (homozygous)