

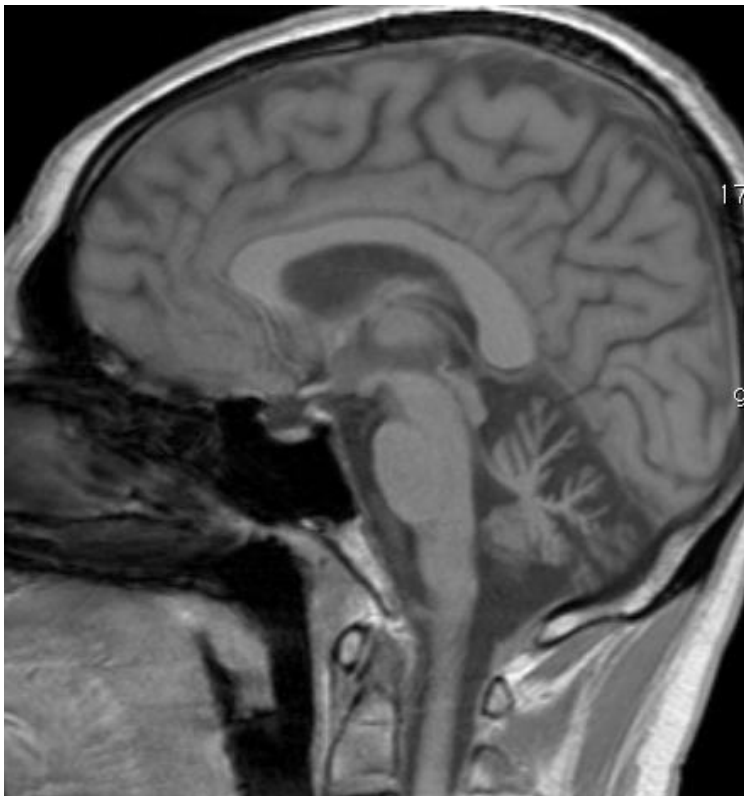
Supplementary information

Orthogonal factors in the disease severity of *ATP1A3* mutations: impairment, misfolding, and allele competition

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ATP1A3 p.Asp742Tyr

Figure S1.



MRI of the adult patient (D742Y) at the time of recent exam showing pronounced cerebellar atrophy.
F. Mochel.

ATP1A3 p.Leu924Pro

T. Luebbert and S. Demarest

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Presentation at birth: The patient was born at 36 2/7 weeks gestation with a birth weight of 2550 gm to a 23 year old G5, P4 mother. His APGAR scores were 7 and 8 after normal spontaneous vaginal delivery less than 18 hours after rupture of membranes. The patient's mother was positive for chlamydia trachomatis during pregnancy but was treated adequately with penicillin. Gestation was otherwise complicated by preterm labor starting at 6 months, maternal hypertension treated with nifedipine, and maternal hypothyroidism treated with levothyroxine.

The patient had microcephaly and hovered around the 1st percentile throughout his course. He was noted at birth to have multiple dysmorphisms: a small anterior fontanelle, posteriorly sloping forehead with temporal extension of hairline, slightly up-slanting and short palpebral fissures, tented upper lip, facial asymmetry especially noted with crying, but notable at other times as well. He had a tendency to hold the left forearm externally rotated with flexed hand (but no contracture noted and able to supinate); IV on right so posturing not noted on the right; very long fingers with 5th finger clinodactyly and distally placed thumbs bilaterally; normal creases; long feet with sandal gap and deep plantar crease between first and second toes bilaterally; normal nails. The dysmorphic findings were ultimately subtle and nonspecific. In particular, they were less apparent as he got older.

On day of life 3, the patient had several episodes of apnea† preceded by crying. Some of these episodes resolved spontaneously and others required stimulation to resolve. He went home from the hospital, had no episodes from day of life 9 through 13, then was admitted to the neonatal intensive care unit after episodes recurred. A chromosomal microarray was sent, and the patient underwent electroencephalography as described below.

†Often observed in severe *ATP1A3* cases.

Electroencephalography: Initial continuous electroencephalogram (EEG) at day of life 16 captured one apneic episode, which correlated to an episode of bradycardia to 84 bpm but was not accompanied by any electrographic changes. He continued to have apneic spells, and it was eventually noted that he lacked suck and gag reflexes after an episode. EEG on day of life 36 noted 1) background asynchrony, 2) some multifocal spikes/sharp waves in both hemispheres, maximal in the right temporal region, 3) some positive sharp waves in multifocal regions, maximal in the right temporal region, 4) mildly excessive background discontinuity, 5) three focal seizures possibly without dyscognition, and 6) concern for a cluster of epileptic spasms. Spasms were not seen on subsequent EEGs.

EEG on day of life 49 was notable for continuous, synchronous background while awake and asynchronous background during sleep. There continued to be frequent epileptiform discharges in the right hemisphere. Repeat EEG on day of life 69 revealed that the background was continuous and synchronous during both wakefulness and sleep. Around this time, he had a prolonged apneic event, and EEG revealed focal status epilepticus from the right temporal lobe. Subsequent EEGs continued to note frequent right-sided slowing and epileptiform discharges. At approximately one year of life, the patient was admitted for epilepsy monitoring unit characterization of spells, where the epileptiform discharges were predominant on the left side.

Relevant Laboratory studies: AASA and pyridoxyl-6-phosphate testing for pyridoxine-dependent seizure were normal.

Imaging: PET scan of the brain was completed at 2.5 months of age. This revealed possible relative hypermetabolism in the right posterior insula and perisylvian region when compared to the contralateral side. MRI brain without contrast was completed at one month of age and was normal.

Developmental delay: From a development perspective, he was profoundly hypotonic with persistent head lag through 2 years of age. He did not sit independently, roll, or walk. He had roving eye movements and poor fix and follow, suggestive of cortical visual impairment. At times, examiners saw that he had asymmetric tone or arm posture, and occasionally facial asymmetry. This was not persistent or stereotyped but could last several hours or days suggesting an alternating hemiplegia-like pattern. This did not manifest until around a year of age.

Family history: Family history was notable for a single febrile seizure in the patient's sister. The patient's brother died at 5 days of life after complications of colon malrotation/volvulus but had no other medical concerns.

Genetic Testing: Infantile epilepsy via GeneDx 53-gene panel was normal at one month of age. Chromosomal analysis (Colorado Genetics Laboratory): normal 46,XY male. Fluorescence in situ hybridization (FISH) for trisomy (Colorado Genetics Laboratory): normal. Comprehensive chromosomal microarray: 575 kb gain in 1q23.3 (paternally inherited), which was thought *not* to be pathologic as the father was non-dysmorphic and seizure-free (lifelong healthy). Whole-exome sequencing (Ambry Genetics, www.ambrygen.com) revealed a missense variant on chromosome 19q13.2 c.277IT>C, Leu924Pro). It was confirmed to be de novo, and to be absent from two developmentally-normal sisters. The same laboratory provided guidance about the likelihood that the mutation was pathogenic. Although not previously described, this mutation was predicted to cause disease given reports of nearby residue changes causing pathologic changes, the absence of this mutation in healthy family members, the absence of this mutation in variant databases, predictions to be deleterious by in silico models SIFT and PolyPhen2, and the conservation of the structure of this domain throughout vertebrates and especially mammals.

Treatment/response and death: The patient was initially started on Topiramate, then went on phenobarbital, and clobazam was added. The phenobarbital was discontinued with concern that it was making apneic episodes more severe. He went on levetiracetam, clobazam, topiramate, and lacosamide, ultimately being on four anti-seizure medications but continued to have frequent seizures and apneic episodes. As a result, he was not able to be safely discharged from the neonatal intensive care unit for the first several months of life. Given the persistent focal features on EEG that mostly lateralized to the right hemisphere he was discussed in epilepsy surgery conference as a candidate for a right hemispherectomy, which was ultimately rejected. He was eventually discharged home in a palliative care program to maximize comfort. Curiously his apneas abated for more than a year. He died at 2 years, 1 month of age during an apparent apneic event following a seizure suggesting SUDEP, Sudden Unexplained Death in Epilepsy.

ATP1A3 p.Arg463Cys.

I.U. Haq

History. The patient is a right-handed Caucasian man who developed a right leg tremor at age 61. A right-hand rest tremor appeared shortly afterwards. Two days after tremor onset he developed a sustained flexion of his right ankle, foot, toes, and the fingers of his right hand. He had not changed his routine, experienced any chemical exposures, or imbibed alcohol. His dystonia fluctuated in severity but grew more painful over the ensuing month. His right foot began 'balling up,' impeding his ability to walk. Two months after onset he noted episodic spasmodic dysphonia without difficulty swallowing. Other symptoms included migraine headaches beginning in the back of the neck and terminating in the eye, and sometimes accompanied by a bright line in his field of vision. He also reported mild

concentration and memory deficits. Two months after symptom onset he was referred to the movement disorder clinic.

Imaging. Noncontrast MRI of the brain was normal except for small bilateral middle cerebral artery trifurcation aneurysms, confirmed by CT angiogram. He had a dopamine-transporter tagged Ioflupane scan (DaT scan) performed three years after symptom onset. This showed a bilateral but asymmetric uptake of tracer, more reduced on the left than on the right.

Presentation. Neurological examination at his initial visit was significant for mild symmetric hyperreflexia without Babinski signs. He was hypophonic with mild dysphonia. He demonstrated a typical parkinsonian 6-8 Hz resting tremor in his right arm and hand. Dystonic symptoms included bilateral restriction of neck motion (worse on looking leftwards) and posturing of the right toes. His UPDRS-III score was a 43 in the off-medication state. He had some mild cognitive dysfunction, 25/30 on the Montreal Cognitive Assessment (MOCA). He reported subjective benefit on medication and showed an objective dose-dependent improvement of his UPDRS score on carbidopa/levodopa (a 50% reduction with a 200 mg dose).

Two years after symptom onset his symptoms had worsened subjectively but remained fairly constant on formal evaluation (UPDRS-III of 43 in 2009, 46 in 2011). At his four year visit his MOCA had also worsened to a 20/30. Over this time, he experienced increasing neck flexion with moderate anterocollis, bilateral splenius capitis and trapezius hypertrophy, and worsened posturing of his left leg and foot. He continued to have a moderate tremor limited to the right hand and leg. His exam was otherwise normal.

Treatment/response. The patient was treated with carbidopa/levodopa 25/100. He reported subjective benefit on-medication and showed dose-dependent improvement in his UPDRS score on carbidopa/levodopa at his initial visit (a 50% UPDRS III reduction with a 200mg dose. He remained levodopa responsive four years after onset, but showed only a 22% response to a 200mg levodopa dose.

Genetics: Given the time course of his symptoms and the mild dystonic features on examination we obtained testing for DYT1 and RDP. Tests were negative for a GAG946 deletion in the DYT1 gene. An *ATP1A3* gene variant was found in exon 11, c.1387C>T, p.Arg463Cys (rs150785666).

Neuropsychological testing: The patient completed an assessment battery¹ which included subtests from the Wide Range Assessment of Memory and Learning, Second Edition (WRAML-2), Controlled Oral Word Association (COWA), Trail Making (A&B), and Symbol Digit Modalities Test (SDMT). Trail Making and COWA scores were derived from Halstead-Reitan neuropsychological norms. The testing was discontinued due to fatigue and he returned 2 months later to complete the exam. The patient completed the immediate recall trial for Design Memory of the WRAML-II on the first day. All other tests were completed on the second visit. Results are detailed in **Table S1**. The scores were in line with results from RDP patients tested with the same battery.¹

Discussion. This patient displayed several aspects of the established RDP phenotype: rapid-onset dystonia, static dystonic symptoms, and dysarthria and hypophonia with mild dysphagia, and also mild cognitive dysfunction. He differed from the typical RDP patient in several important respects. His age of onset was later than in most kindreds (prior to 40 years of age in 94% of patients). Most significantly, he presented with the unilateral levodopa-responsive 6-8 Hz resting tremor typical of idiopathic Parkinson's disease. His response to dopaminergic therapy was dose dependent. There was a suggestion of selective symptom response: levodopa administration (at a 200mg dose) relieved his tremor and substantially improved his bradykinesia while leaving his rigidity and dystonia substantially unchanged.

Table S1. Neuropsychological data

| Test | Score* | Percentile | Descriptor** |
|---|--------|------------|-----------------|
| Memory (WRAML-II) | | | |
| Picture Memory Scaled Score | 12 | 75 | Above Average |
| Picture Memory (Delayed) Scaled Score | 11 | 63 | Average |
| Verbal Learning Scaled Score | 4 | 2 | Mild Impairment |
| Verbal Learning (Delayed Recall) Scaled Score | 4 | 2 | Mild Impairment |
| Verbal Learning (Delayed Recognition) Scaled Score | 5 | 5 | Borderline |
| Design Memory Scaled Score | 5 | 5 | Borderline |
| Executive Functions | | | |
| Trailmaking A T-score | 51 | 55 | Average |
| Trailmaking B T-score | 47 | 40 | Average |
| COWA (Linguistic) T-Score | 37 | 10 | Mild Impairment |
| COWA (Semantive) T-Score | 52 | 59 | Average |
| SDMT (Written) Z-Score | -2.00 | 2 | Very Low |
| SDMT (Oral) Z-Score | -3.00 | 1 | Very Low |

*T-scores derived from demographically adjusted (age, gender, ethnicity, and education) neuropsychological norms.

** Descriptors as per scoring manuals for neuropsychological tests

1. Cook, J.F. et al. Cognitive impairment in rapid-onset dystonia-parkinsonism. *Mov. Disord.* **29**, 344-350 (2014).