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Supplemental Data

Variants in the Oxidoreductase PYROXD1 Cause Early-Onset Myopathy with Internalized Nuclei and Myofibrillar Disorganization

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Case summaries

Family A

A-II1 and A-II2 were affected brothers born to a non-consanguineous Australian family. They had one unaffected sister.

Both brothers had normal early gross motor milestones, walking by 12 months of age and were able to run. Mild weakness became evident from 5 years of age in the older brother and from 8-9 years of age in the younger. Both boys had a non-progressive course up until their late teens, followed by gradually progressive weakness. At 21 years of age the older brother was able to walk an unlimited distance, but had difficulty on stairs. At 29 years he was able to walk slowly for several hundred metres with the support of a cane. He was not able to climb stairs. The younger brother was able to walk independently at 26 years, but was increasingly reliant on a handrail to climb stairs.

Both boys had swallowing difficulties and nasal speech. Symptoms were more pronounced in the younger brother who had a history of nasal regurgitation and required surgery to his palate and pharyngeal muscles for velopharyngeal incompetence.

The older brother had specific learning difficulties with reading and spelling, but other domains were in the average or superior range. The younger was described as a good student.

Early cardiac examinations were normal. A repeat echocardiogram in A-II1 at 27 years of age showed abnormal septal motion and a low normal ejection fraction. Respiratory function tests showed a mild restrictive pattern which was not progressive.

On examination, both boys were of tall stature (90-97th centile) and had macrocephaly (>97th centile). The older brother in particular was very slim, with reduced muscle bulk. Both boys had distinctive facial features with a tall, broad forehead, synophrys, deep-set almond shaped eyes, a short well-defined philtrum, a hypertrophic nasal tip, thick columella and thick alae, mild micrognathia and slightly pointed chin. Both boys had high-arched palates, crowded dentition, dental malocclusion with an overbite and required orthodontic work. Both had nasal speech.

Joint hypermobility of the DIP, PIP and MCP joints was prominent in the younger brother who also had a history of recurrent partial patella subluxation. The older brother had an inferior pectus excavatum and moderate pes cavus. Achilles tendon contractures were present in A-II1 and

mobility was reduced around the shoulder joint. A-II1 had a mild thoracic scoliosis from 20 years, and both developed mild thoracic kyphosis.

Mild facial weakness was present in both boys. No ptosis or ophthalmoplegia were present. Mild neck flexor weakness was present, without neck extensor weakness. Proximal and distal limb weakness (4- to 4+/5) was present. Both brothers had marked wasting of the intrinsic muscles of the hand, particularly the thenar eminence. Weakness was most severe in A-II2 with abductor pollicis brevis graded 0/5 bilaterally. Reflexes were reduced or absent.

CK was normal in A-II1 and ranged from normal to 1051 in A-II2. Muscle biopsy findings are described in Table 1. Electrophysiologic studies are summarized in Table S2.

Family B

B-II2 and B-II3 were affected brothers born to a consanguineous Turkish family. They had one unaffected brother. The parents were both healthy.

The two brothers had normal motor milestones, walking by 15 months of age and were able to run. Weakness of lower limbs became evident from 10 years of age in both brothers. The weakness progress slowly and concerns the distal and proximal muscles of the four limbs. The two siblings are still fully ambulant but the older brother (B-II3) is able to climb 20 stairs with the banister. The young brother (B-II2) walks independently and can climb 20 stairs without the banister.

On examination, both boys were of tall stature. The young brother was very slim, with reduced muscle bulk. Both boys had facial weakness. Micrognathia and retrognathia and mild ptosis without ophtalmoplegia were observed in the younger brother (B-II2). Both had nasal speech and difficulties in swallowing were noticed in the older brother (B-II3).

Muscle weakness was observed in proximal and distal muscles. Neck flexor weakness was present (MRC score= 3/5). The older brother (B-II3) was more severely affected compared to the younger one (B-II2). MRC score was respectively scored 3/5 and 4/5 in the older and young brother. Both brothers had marked wasting of the intrinsic muscles of the hand, particularly the thenar eminence. Deep tendon reflexes were reduced or absent. Nasal speech and dysphagia were noticed in both brothers. Pes planus was present in both brothers. Bilateral scapular winging was present in both brothers. They had normal cognitive development.

Creatine kinase levels ranged from (500-700 UI/L). Electrocardiogram and echocardiography were normal. Vital capacity was reduced in B-II2 and near normal in the oldest one (B- II3), respectively 66% and 83% of the theoretical value.

Electromyography performed in both brothers showed myopathic patterns and absence of neuropathy. Repetitive nerve stimulation was normal. The results of muscle biopsy are described in Table 1

Family C

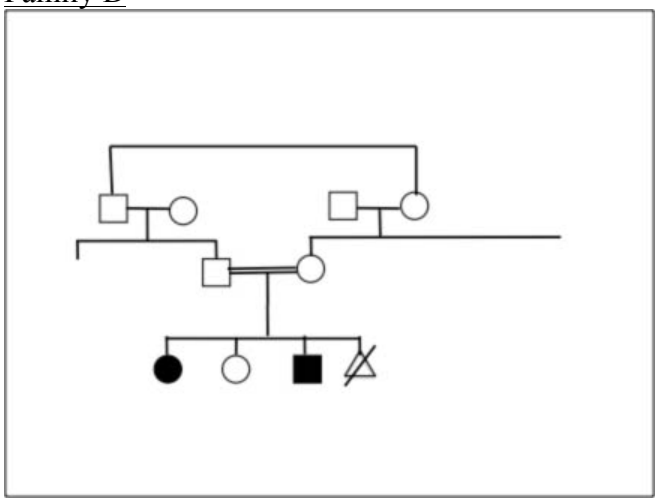
C-II1 is a 9-year-old female of Persian Jewish descent. First concerns arose at birth when she was noted to have hypotonia and poor head control. She gradually improved and motor milestones where at the late side of normal. When she started walking, she was noticed to fall frequently. She was able to very slow make gains, however physical tasks became increasingly difficult at age 6-7 years. She has a history of very thin nails and hair, soft skin (particularly on the ears), and tactile allodynia on the scalp. She was diagnosed with unspecified unilateral hearing loss at age 6 years. Clinical scoliosis was noted at age 6 years. Currently, she fatigues easily, with frequent falls.

Family history is significant for a younger brother with similar symptoms. Consanguinity was denied.

On examination she was found to have diffusely hypotrophic/atrophic muscle mass. There were end grade contractures in the ankles. She had mild incurving of 5th digits bilaterally, mild syndactally of toes 2 and 3, sndmmild incurving of the forth and fifth toes. There was joint laxity in the elbows and fingers but not in the distal fingers. Deep tendon reflexes were +1 at the ankles and brachioradialis, but otherwise absent. Vibratory sense was normal. Spinal examination revealed lumbar lordosis and thoracolumbar rigidity, but no overt scoliosis. There was mild scapular winging. She ambulated without a primary heel strike and mildly stiff gait. She accelerated into a Trendelenberg trot, barely clearing the floor with occasional left foot circumduction. She was unable to dorsiflex to perform heel gait. She arose from the floor into a sitting position by rolling to prone and pushing into a sitting position. She then arose into a standing position by pushing from a quadruped position into an upright kneel, moving to the wall and arising to a standing position with one hand on the bar and a one-handed Gowers'. Manual muscle testing revealed mostly axial weakness, involving both upper and lower extremity around 4+/5 and 4-/5, except for shoulder abd. (3/5), wrist flex (3+/5), neck flex (2/5) and hip abduction (3/5).

C-II2 is her 7-year-old brother. First concerns arose around age 2.5 months when he was noted to have poor head control and hypotonia. He made gradual improvements over time, without any decline. Milestones have been normal. He falls frequently. He has a history of frequent and severe respiratory infections- multiple bronchitis (>25) and pneumonia twice. Upon exam he was found to have diffusely hypotrophic/atrophic muscle mass. He has mild tightness in the knees, and no other contractures were noted. He has syndactyly of toes 2 and 3, and the second toes overlap the third toes bilaterally. There was mild joint laxity in the elbows, wrists and fingers. No laxity was present at the knees or distal fingers. Deep tendon reflexes were +1 at the patella, biceps, and brachioradialis, +2 at the ankle and a trace at the triceps. He had normal vibratory sensation in the distal extremities. Spinal examination revealed no scoliosis but thoracolumbar rigidity. There was mild asymmetric (L>R) scapular winging. He ambulated with a very mild Trendelenberg gait, primarily on his toes. He accelerated into a wide-based, in-toe Trendelenberg trot, not clearing the ground with excessive arm swing. He was unable to jump. He arose from the floor into a sitting position by rolling to prone and pushing back into a sitting position. To arise into standing, he transitioned from a quadruped position into a kneel and arose with a one-handed Gowers' with a second hand brush-off. Manuel muscle testing revealed mostly axial weakness, involving both upper and lower extremity around 4/5 and 4-/5, except for deltoid (3+/5), finger spread (3+/5), neck flex (2/5); hip extension (5-/5).

Family D



Two affected siblings presented with infantile onset muscle weakness. D-II1 is a female aged 22 years and D-II3, her affected brother, is aged 17.5 years. D-II1 walked at 2.5 years and D-II3 at 2 years. Prenatal and natal histories were normal. D-II1 had poor sucking in the newborn period and could not achieve breast-feeding. She tolerated bottle-feeding and did not need a nasogastric tube. Weakness was slowly progressive in both siblings, however they are both still ambulant aged 22

and 17.5 years. They have difficulty running and climbing stairs, with D-II1 more severely affected. Both siblings have swallowing difficulties. D-II1 is morbidly obese (BMI: 41), and D-II-2 is overweight (BMI: 29.3).

On examination, they had symmetrical proximal weakness of the upper and lower limbs, and a positive Gower's sign. Deep tendon reflexes were reduced or absent. There were no joint contractures or scoliosis. The two siblings had mild ptosis, facial weakness, elongated face, high arched palate and nasal speech.

Cognition was normal in both. D-II1 had a history of frequent respiratory infections in childhood. Cardiac evaluations were normal. Creatine kinase levels ranged from normal (290-376 IU/L) to 700 in D-II1 and 400 in D-II3 to 700 IU/L. Electromyography showed myopathic patterns for D-II3. Full results are summarized in Table S.., Electrophysiological studies were not performed in other sibling. Muscle biopsy findings are summarized in Table 1.

Family E

The index case is a 15-year-old girl. She presented with easy fatigue, frequent falls, difficulty in climbing and standing. She had facial weakness, nasal speech and difficulty in swallowing. Her symptoms started at the age of 4-5 years and the course was mildly progressive. She had frequent respiratory infections. Physical examination revealed facial weakness, high arched palate, proximal>distal weakness (4-/5 and 4+/5) with mild distal atrophy (tenar atrophy) and deep tendon reflexes were absent or hypoactive. There were no ophthalmoplegia, contractures, scoliosis or rigid spine. She had normal mental development and cognitive functions.

Parents are 3rd degree consanguine. Her brother is 17-years-old and healthy, mother had no symptoms, the father had a diagnosis of ankylosing spondylitis.

Laboratory tests:

CK level was normal (43 IU/L).

EMG showed myopathic changes. Sensory nerve conduction studies were normal.

Motor conduction studies revealed low amplitude responses in the right ulnar and tibial nerves, and no response in right median and peroneal nerves. Needle EMG studies showed low amplitude, short time, normal and polyphasic motor unit potentials which is more prominent in lower extremity muscles. EMG findings were consistent with myopathic changes.

Muscle biopsy was performed at the age of 10 and prominent histopathologic features were

hypertrophic and atrophic fibers, predominance of type 1 fiber types, central nuclei in most of the fibers, "whorling" in occasional fibers, enlargement of the interstitial adipose tissue and perimysial connective tissue. Oxidative enzyme stains showed irregular staining in hypertrophic fibers and some of the fibers had reduced enzymatic activity in the central regions.

Summary of HPO terms

HP:0001324 9/9; HP:0001265 9/9; HP:0002058 9/9; HP:0001611 9/9; HP:0000218 7/9; HP:0002015 7/9; HP:0003691 5/9; HP:0001763 5/9; HP:0001382 4/9; HP:0000276 4/9; HP:0040081 4/9; HP:0002783 4/9; HP:0000508 3/9; HP:0001771 2/9; HP:0009183 2/9; HP:0002650 2/9; HP:0003306 2/9; HP:0030193 2/9; HP:0000767 2/9; HP:0000689 2/9; HP:0002091 2/9; HP:0000308 1/9; HP:0000577 1/9; HP:0001761 1/9; HP:0001653 1/9; HP:0000602 0/9

Table S2 Electrophysiological characteristics of individuals with *PYROXD1* myopathy

ID	Family A-II1			Family A-II2			Family B-II2			Family B-II3			Family C-II1			Family C-II2			Family D-II3			Family E-II2					
Age at investigation	29 years[1]			26 years[1]			26 years			16 years			7 years			5 years			17 years			8 years					
***PYROXD1* variants**	c.285+1G>A c.116G>C, p.Q372H			c.285+1G>A c.116G>C, p.Q372H			Hom. p.N155S	c.464A>G,		Hom. p.N155S	c.464A>G,		c.414+1G>A c.464A>G, p.N155S			c.414+1G>A c.464A>G, p.N155S			Hom. p.N155S	c.464A>G,		c.464A>G, p.N155S c.1159_1160insCAAA					
Motor Nerve Conduction																									**Normal values**		
Nerve and site	Lat. (ms)	Amp. (mV)	C.V. (m/s)	Lat. (ms)	Amp. (mV)	C.V. (m/s)	Lat. (ms)	Amp. (mV)	C.V. (m/s)	Lat. (ms)	Amp. (mV)	C.V. (m/s)	Lat. (ms)	Amp. (mV)	C.V. (m/s)	Lat. (ms)	Amp. (mV)	C.V. (m/s)	Lat. (ms)	Amp. (mV)	C.V. (m/s)	Lat. (ms)	Amp. (mV)	C.V. (m/s)	Lat. (ms)	Amp. (mV)	C.V. (m/s)
Median (R)																											
APB-Wrist	4.5	**2.0**		5.5	**0.1**		4	**4.45**		3.5	5.4		3.0	**4.9**		2.4	**3.6**					NR	NR		≤3.6	≥5	
Elbow	9.1	**1.8**	50	NR	**NR**		9.1	**4**	57	8.1	5	56	6.2	**4.5**	50	**5.2**	**3.9**	45									≥48
Axilla																											
FPL-Elbow*	3.9	**0.9**		4.6	**1.0**																						
Axilla*				8.8	**1.2**	60																					
Ulnar (R)																											
ADM-Wrist	3.9	**1.0**		3.3	**0.2**		3.3	**2.58**														5.8	**0.6**		≤3	≥5	
Below elbow	8.3	**0.9**	61	8.0	**0.2**	55	8.3	**2.60**	56													8.5	**0.2**	57.4			≥48
Above elbow	10.4	**0.9**	48				10.4	**2.64**	46																		
Common peroneal (R)																											
EDB-Ankle	**NR**	**NR**		**NR**	**NR**		5	**0.61**		4.5	**1.20**								**3.85**	**1.5**		**NR**	**NR**		≤5	≥3	
Fibular head							12	**0.53**	44	11	**1**	46							10.75	4.0	53.3	**NR**	**NR**				≥42
Tibial (R)																											
AHB-Ankle				8.7	**0.1**	-	7.3	**3.97**	-	5.1	9.80	-							**4.45**	12.7		**3.8**	**1.9**		≤5.5	≥4	
Knee																			14.15	9.9	43.3	10.0	**1.4**	44.4			≥42
Spinal accessory					**1.1**																					2- 4.2	
Trapezius																											
Sensory Nerve Conduction																									**Normal values**		
Nerve and site	Onset Lat. (ms)	Amp. (μV)	CV (m/s)	Onset Lat. (ms)	Amp. (μV)	CV (m/s)	Onset Lat. (ms)	Amp. (μV)	CV (m/s)	Onset Lat. (ms)	Amp. (μV)	CV (m/s)	Peak Lat. (ms)	Amp. (μV)	CV (m/s)	Peak Lat. (ms)	Amp. (μV)	CV (m/s)	Onset Lat. (ms)	Amp. (μV)	CV (m/s)	Onset Lat. (ms)	Amp. (μV)	CV (m/s)	Onset Lat. (ms)	Amp. (μV)	CV (m/s)
Median (R)																											
Orthodromic Digit II	2.8	15		3.0	11		3.8	25	50	3.2	28	48	2.4	123	51	2.2	55	54				2.3	63	51.9	≤4	≥10	≥48
Ulnar (R)																											
Orthodromic Digit V	2.7	8		2.5ms	10		3	13	60													1.92	42	58.1	≤3	≥8	≥48
Sural (R) antidromic	5.4	**5**		7.1ms	**5**		2.3	33	42	1.9	26	42	ND	ND		ND		ND	4.1	8.7	51.3	3.0	21	52.2	≤3	≥10	≥40
Sural (L) antidromic	ND	ND		6.9ms	**2**		2.2	35	43	2.1	28	40													≤3	≥10	≥40
Repetitive Nerve Stimulation																											
				Normal																							
Electromyography																											
	Myopathic			Myopathic			Distal and proximal muscles myopathic			Distal and proximal muscles myopathic			Limited study. Vastus lateralis and tibialis anterior myopathic			Limited study. Myopathic (vastus lateralis only)			Myopathic			Myopathic legs >arms					

APB, abductor pollicis brevis; ADB, abductor digiti minimi; EDB, extensor digitorum brevis; AHB, abductor hallucis brevis; NR, No response; ND, Not done; Lat, Latency; Amp, Amplitude; CV, conduction velocity
* To flexor policis longus (FPL) – non-standard median study more proximally (normal values: FPL-Elbow >2.5mV)

Table S3 Haplotype Analysis
Position of key variants on Chr12 (including and flanking the N155S variant) defining a region consistent with a common Turkish haplotype in Families B, D and E.

Chr12 start/end position (bp)	**N155S Turkish haplotype sequence**
21331987	C
21590788	A
21593404	C
21600953	A
21605064	G (N155S)
21608891	C
21609127	C
21609684	G
21609733	G
21609761	AACT
21609907	A
21609936	G
21614142 - 21614146	-
21615664	C
21621737	G
21624309	-
21628320	T
21628336	A
21628791	C
21628812	A
21629987	C
21629993	C
21644666	C
21654831	G
21657591	T
21659973	C
21661508	G
21665207	G
21679911	A
21680609	G