The American Journal of Human Genetics, Volume *94*

Supplemental Data

Mechanism, Prevalence, and More Severe Neuropathy Phenotype of the Charcot-Marie-Tooth Type 1A Triplication

Pengfei Liu, Violet Gelowani, Feng Zhang, Vivian E. Drory, Shay Ben-Shachar, Erin Roney, Adam C. Medeiros, Rebecca J. Moore, Christina DiVincenzo, William B. Burnette, Joseph J. Higgins, Jun Li, Avi Orr-Urtreger, and James R. Lupski

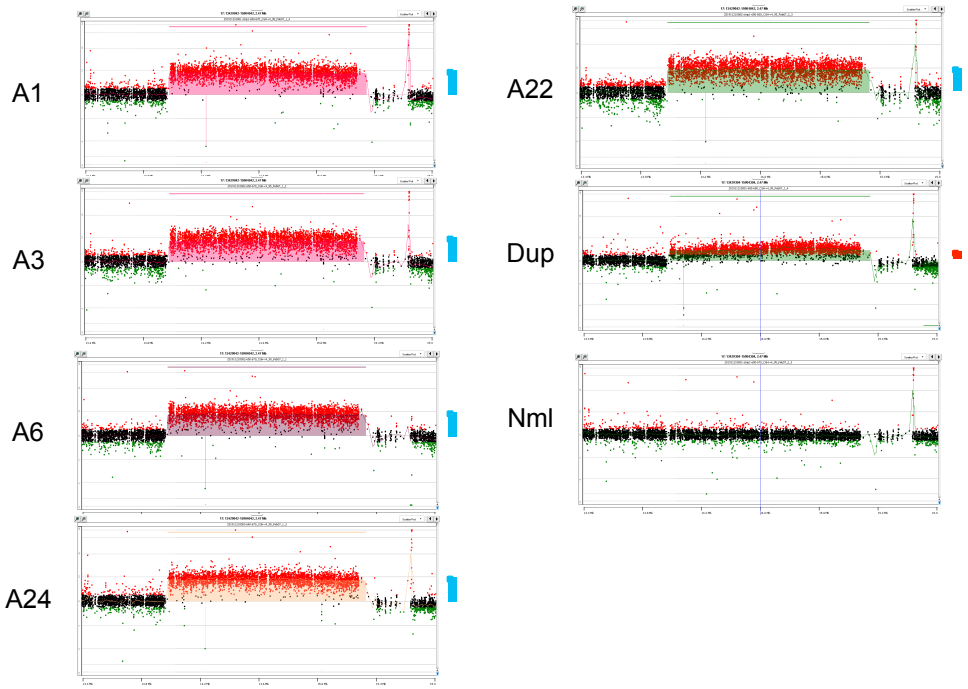

Figure S1. Anonymized subjects with CMT1A triplication identified from the diagnostic screening. Subjects with an MLPA data suggestive of CMT1A triplication are subject to array CGH analysis. Results are consistent with them having four copies of the CMT1A region, though the possibility of homozygous duplication cannot be ruled out. Array results showing a CMT1A duplication and normal copy number are listed at the lower right corner as a comparison. The red bar next to the duplication result indicates a gain in copy number. The blue bars indicate a further gain in copy number, consistent with triplication.

Table S1. Nerve conduction studies and other clinical descriptions

ID	Age	Sex	Ulnar Motor			Median Motor			Radial Sensory		
			Distal Latency (mS)	NCV (m/S)	CMAP (mV)	Distal Latency (mS)	NCV (m/S)	CMAP (mV)	Distal Latency (mS)	NCV (m/S)	CMAP (mV)
BAB3328	19	M	9.1	7.1	1.2	13.6	8.2	2.4	NR	NR	NR
KSLII1	65	F	8.5	28	2.9	15	28	2.6	NR	NR	NR
KSLII2	60	F	2.9	62	9	3.6	58	6.1	3.3	45	14
KSLIII1	39	M	2.8	59	10.7	4.9	55	13.1	2.5	58	14
KSLIII2	36	F	8.7	24	6.4	11.9	31	7	NR	NR	NR
KSLIV5	4	M	NT	NT	NT	8.7	15.6	2.6	NT	NT	NT

NR, no response; NT, not tested; m/S, meters/second; mV, milliVolts; mS, milliSeconds.

For BAB3329, only the lower limbs were examined: distal motor latencies in common peroneal 7.6, tibial 8.5, conduction velocity in the common peroneal 26.2, tibial 23.2, sensory responses were not tested. The exam was very limited due to extreme discomfort.

In family #1, the index patient is a 29-year-old Jewish Ashkenazi male. Clinically this patient was affected since early childhood. Bilateral club feet were recognized at the age of a few days, and were treated by casts. Waddle gait was observed while he started walking and then some clumsiness was observed during walking. He had kyphoscoliosis. No signs of muscle atrophy and/or contractures were reported at the age of 8 and 12 years. Difficulties in running appeared at the age 13y and difficulties while climbing stairs were

first reported at 15y. Orthotic assist devices (ankle foot orthoses; AFO) were used since age 16y to stabilize steppage gait. Since age 18y he also had distal weakness of the hands. Flexion contractures of fingers appeared at the age of 23y with a progressive course. When examined at 23y, he walked with difficulty using bilateral AFOs and dragging of his feet. Muscle strength was 5/5 proximal in the arms, 4/5 in iliopsoas, 2/5 in quadriceps, 0/5 distal in hands and feet consistent with DSP. He had severe sensory loss in the feet and hands with distal predominance, areflexia, extremely severe atrophy of all limbs, flexion contracture of the fingers. No respiratory deficit was observed. When examined at 29y, he had severe muscle atrophy of lower limbs>upper, distal>proximal, contractures, very high feet arches (Figure 1). MRI of lumbar spine at age 16y showed hypertrophy of fibers of cauda equina. Nerve conduction studies (NCS) at age 8y revealed a mainly demyelinating peripheral neuropathy. Repeated examination at 19y detected a significant progression of these findings (Table S1). NCS at 23y revealed lack of motor responses of the right median nerve, both tibial nerves and common peroneal nerves. EMG showed signs of denervation in all lower limb muscles and chronic neurogenic damage of upper limb muscles, more severe distally. His sister at age 20 has very mild foot drop bilaterally and atrophy of first dorsal interosseous muscles (FDI). Her NCS is compatible with a mild demyelinating motor/sensory polyneuropathy. His mother at age 52 has numbness of feet and atrophy of FDIs only. His maternal grandmother, at age 75, does not have any neuromuscular complaints.

In family #2, the index patient is a four-year-old boy who presented to the clinic for evaluation of unsteady gait, frequent tripping and falling. He began walking at 15 months

of age and was first noted to have symptoms of weakness of his distal legs at 3y. When he was examined at 4 years of age, he was noted to have moderate bilateral *pes cavus* and mild distal leg atrophy, with mild-to-moderate weakness in ankle dorsiflexion, eversion, and great toe extension. Tendon reflexes were absent. Detailed sensory examination could not be accomplished due to the patient's age. Nerve conduction studies (NCS) showed features of a primarily demyelinating polyneuropathy with significantly prolonged distal latencies and severely reduced conduction velocities (Table S1). The mother of the patient is a 36-year-old woman with high arches. She was clumsy in playing sports since childhood. NCS revealed significantly prolonged distal latencies and uniform slowing of conduction velocities (Table S1). The father, maternal uncle and maternal great aunt of the patient are asymptomatic. The maternal grandmother of the patient was examined at 65 years of age. She walked at normal age, played basketball in the 8th grade. However, she reported being a slow runner, tripping often and frequent falls. She was reluctant to participate in physical education classes. High arched feet were noted in her teenage years. With respect to her hands, she has had problems opening jars or buttoning. She is still able to cut food. She denied any problem sensing temperature or pain. Occasionally, she feels "electric shock" like sensation in her feet or legs. At the present, pain in the bottom of her feet has been constant. When she walks, she tends to back her knee joints. She has developed spine scoliosis for decades. On examination, she was found to have *pes cavus* and hammer toes. No weakness was noted on motor examination, while tendon reflexes were absent. Vibratory sensitivity was reduced distal to the knees. NCS revealed significantly prolonged distal latencies, uniform conduction slowing, reduced motor amplitudes, and absent sensory responses (Table S1).

Table S2. Primer sequences for microsatellite analysis

Primers	Sequence	Repeat Unit #
AFMA070TC5-F	[FAM]-GAAGTTTACGAAAATTGCTGTC	Di-
AFMA070TC5-R	GTGTCTT AAGGGTAGTTCTGCGTGC	
AFMB336ZG9-F	GTGTCTT TACAGTTTCTTGTGTGCCC	Di-
AFMB336ZG9-R	[FAM]-AGTAACTCTGAGGACTTGCTCAT	
AFMB355ZF5-F	[FAM]-AGCTGAGATGGTGCCACTG	Di-
AFMB355ZF5-R	GTGTCTT TCATGGAAGACAATTTTGCG	
D17S2220-F	[FAM]-CCTCAGTCATCTTTCTCCTT	Tetra-
D17S2220-R	GTGTCTT TGGGCAACAGAGCAAAATCC	
D17S2224-F	GTGTCTT GTTCATTCTATCGTCTCAAA	Tetra-
D17S2224-R	[FAM]-AAGGCTACCATAAATCTTGT	
D17S2226-F	[FAM]-GCATTCTTGTCTCAGTCCTG	Tetra-
D17S2226-R	GTGTCTT CCAGAGCTAACACCACATTC	
D17S2227-F	[FAM]-TTAAACTAGCATTCTTCCAA	Penta-
D17S2227-R	GTGTCTT TAACCAGTTTCATCTCACAG	
D17S2228-F	[FAM]-GGCTGTCATAAATGTTCCTA	Tri-
D17S2228-R	GTGTCTT AGGTAAAGGTTCTGGTGAGC	
D17S2229-F	[FAM]-CCCATTCCATAGTCATCAGA	Di-
D17S2229-R	GTGTCTT TGCCATTTTACCACAAGAGG	
D17S4A-F	[FAM]-CTACTTGCATATGCACTTTC	Tetra-
D17S4A-R	GTGTCTT GCACTAAAGTAGCTTGTAAC	
CMT-STR-8F	[FAM]-CTGCCACTGCACTCACTCTA	Tetra-
CMT-STR-8R	GTGTCTT AGAAGGGACTCAATGCATACT	
D17S2230-F	[FAM]-GGAAACTGATGTCTAAAACT	Penta-
D17S2230-R	GTGTCTT GTGAATCCAGGAGGCAGAGC	
CMT-STR-5F	[FAM]-AGTTAGCAGAGGTCGCACCAA	Tetra-
CMT-STR-5R	GTGTCTT AGGTGGGCTTCACATGGTAGA	
AFM240XE5-F	GTGTCTT AGCTCACTGTAGCCTATCCTC	Di-
AFM240XE5-R	[FAM]-AAATGCAGAGTCAAACTTGTAGA	
AFM347XA9-F	[FAM]-CGTGGCCTATTATGAGACTTC	Di-
AFM347XA9-R	GTGTCTT GATTTAGGTAGGGTTCTCCAG	

Table S3. Common recurrent CMT1A duplications and triplications detected by clinical MLPA screening

	2012	**2011**	**2010**	**2009**	**2008**	**2007**
Number of total assays	**2656**	**2977**	**3147**	**3581**	**4085**	**4215**
Number of duplications	**393**	**429**	**455**	**454**	**474**	**547**
Number of triplications	**1**	**0**	**1**	**0**	**2**	**2**