

Supplemental Data

Expansion of Human-Specific GGC Repeat

in Neuronal Intranuclear Inclusion

Disease-Related Disorders

Yun Tian, Jun-Ling Wang, Wen Huang, Sheng Zeng, Bin Jiao, Zhen Liu, Zhao Chen, Yujing Li, Ying Wang, Hao-Xuan Min, Xue-Jing Wang, Yong You, Ru-Xu Zhang, Xiao-Yu Chen, Fang Yi, Ya-Fang Zhou, Hong-Yu Long, Chao-Jun Zhou, Xuan Hou, Jun-Pu Wang, Bin Xie, Fan Liang, Zhuan-Yi Yang, Qi-Ying Sun, Emily G. Allen, Andrew Mark Shafik, Ha Eun Kong, Ji-Feng Guo, Xin-Xiang Yan, Zheng-Mao Hu, Kun Xia, Hong Jiang, Hong-Wei Xu, Ran-Hui Duan, Peng Jin, Bei-Sha Tang, and Lu Shen

Supplemental Note

Case Report

Case F1-IV: 6

A 47-year-old male presented with limb weakness for 14 years. His four brothers also presented with muscle weakness in their early 30s. Muscle weakness began in the distal portion of the lower limbs, then moved up to the throat muscles and face. He routinely felt numbness in his limbs. The neurological examination revealed miosis, muscle atrophy, bilateral hand resting tremor, postural tremor, intention tremor, ataxia and weakness of the four limbs. Mini-Mental State Examination (MMSE) score was 29. Nerve conduction studies showed slowed motor and sensory nerve conduction velocity. Eosinophilic intranuclear inclusions were found in his skin samples, and the diagnosis of NIID was made.

Case F6-II: 3

A 49-year-old male was admitted to the hospital because of progressive slow walking over the last 3 years. The man was diagnosed with Parkinson's disease in 2017, and showed good response with dopaminergic therapy. He also complained about sleep disorders, constipation, and urgency of urination. The neurological examination revealed muscle rigidity and bradykinesia of right upper and lower limb. Unified Parkinson's disease rating scale (UPDRS) motor score was 5. Memory, orientation, and calculation were normal. The Mini-Mental State Examination (MMSE) score was 30, and the Montreal cognitive assessment scale (MoCA) score was 25. Blood chemistry was normal. Further skin biopsy confirmed the diagnosis of NIID.

Case F9-II: 6

An 81-year-old male presented with progressive cognitive decline for 10 years, and was diagnosed with Alzheimer's disease in 2015. His medical history included hypertension, diabetes, and he had to wear a catheter at all times due to urine retention over the past 10 years. His siblings had also been diagnosed with Alzheimer's disease. He had experienced several loss of consciousness episodes that lasted a few minutes each. The neurological examination revealed cognitive impairment, miosis, bilateral hand resting tremor, muscle rigidity, and bradykinesia. His brain DWI images showed typical high signal in the corticomedullary junction, Fluid-attenuated inversion

recovery (FLAIR) images showed severe white matter hyperintensity. CSF examination was normal, including the protein level and cytology. Electroencephalogram showed an increase in slow waves and no epileptiform discharges. He further received a skin biopsy which confirmed the diagnosis of NIID.

Case SP1

A 69-year-old male with left hemiplegia was admitted to the hospital. There was no medical history or family history for him. The neurological examination revealed complete recovery from the left hemiplegia. MMSE score was 27. No typical high signal in acute cerebral infarction was observed in DWI, while symmetrical hyperintense linear lesions was presented in the corticomedullary junction. Nerve conduction studies showed slowed motor and sensory nerve conduction velocity. His skin samples indicated typical NIID pathology.

Figure S1. Summary of Reads Covering Expanded Repeat Region from Oxford Nanopore Platform.

ID	Reads	Insertion Size	Repeat Number	Repeat Pattern	Match Ratio (observed read/pure repeat)
F1-IV:7	read 1	666	222	GGT	77%
	read 2	645	215	GGT	74%
F1-IV:15	read 1	344	114	GGT	88%
	read 2	340	113	GGC	78%
F2-II:3	read 3	295	98	GGC	86%
	read 4	370	123	GGT/GGC	81%/78%
	read 1	561	187	GGC	74%
	read 2	568	189	GGC	71%
	read 3	625	208	GGC	73%
F4-II:2	read 4	628	209	GGC	83%
	read 5	635	211	GGC	76%
	read 1	322	107	GGT/GGC	80%
	read 2	327	109	GGT/GGC	79%/77%
	read 3	384	128	GGC/AGC	87%/83%
F5-II:1	read 4	410	136	GGC	76%
	read 5	357	119	GGC	83%
	read 6	342	114	GGC	84%
	read 1	342	114	GGC	84%
	read 2	263	87	GGC	84%
F5-II:4	read 3	248	82	GGC	87%
	read 1	311	103	GGT/GGC	86%/84%
	read 2	348	116	GGT	88%
F9-II:6	read 1	345	115	GGC	78%
	read 2	396	132	GGC	85%
	read 3	403	134	GGC	78%
	read 4	449	149	AGC	81%
	read 5	356	118	GGC	82%
	read 6	385	128	GGC	77%
	read 7	343	114	GGT/GGC	77%/84%

Raw sequence data of expanded repeat region from Oxford Nanopore platform.

F1-IV:7-read 1

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F1-IV:7-read 2

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F1-IV:15-read 4

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F2-II:3-read 3

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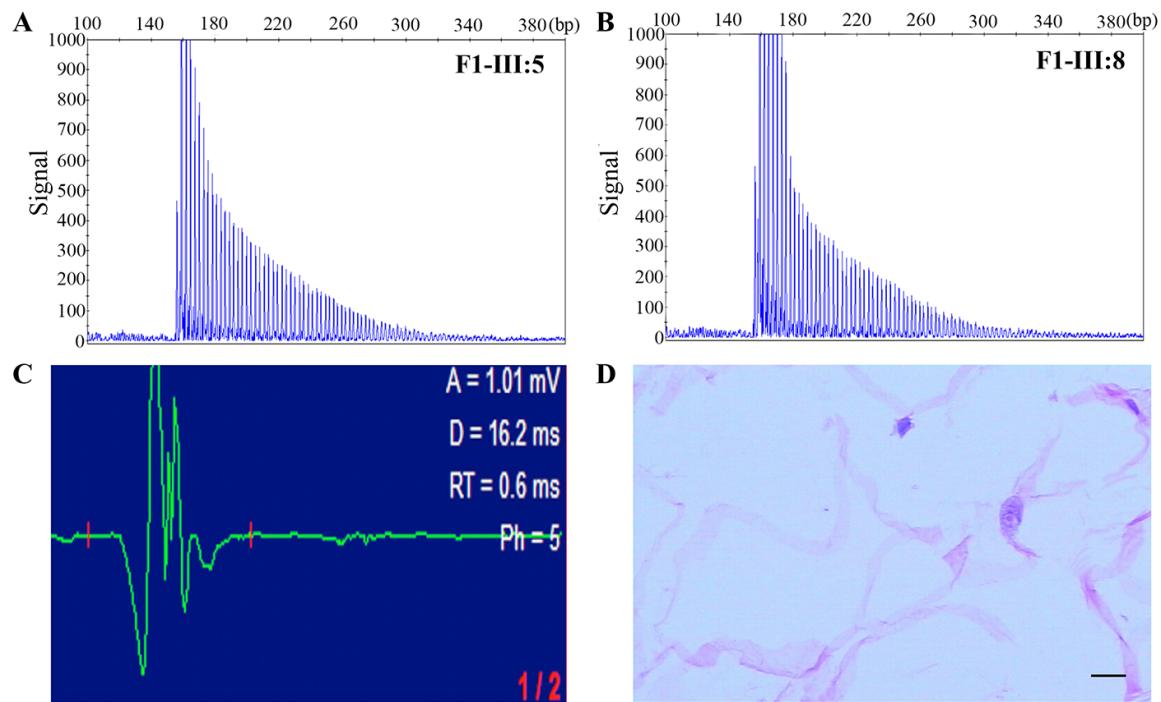


Figure S2. Additional Molecular and Clinical Diagnosis of Family 1. (A-B) RP-PCR assay and GC-PCR assay showed abnormal repeat expansion in subject F1-III:5 (A) and F1-III:8 (B). (C) Representative motor unit potential (MUP) of right tibialis anterior from electromyogram of subject F1-III:8 indicated neurogenic impairment. (D) Skin biopsy sample of subject F1-III:8 displayed typical eosinophilic intranuclear inclusions. Scale bars, 10 μm .

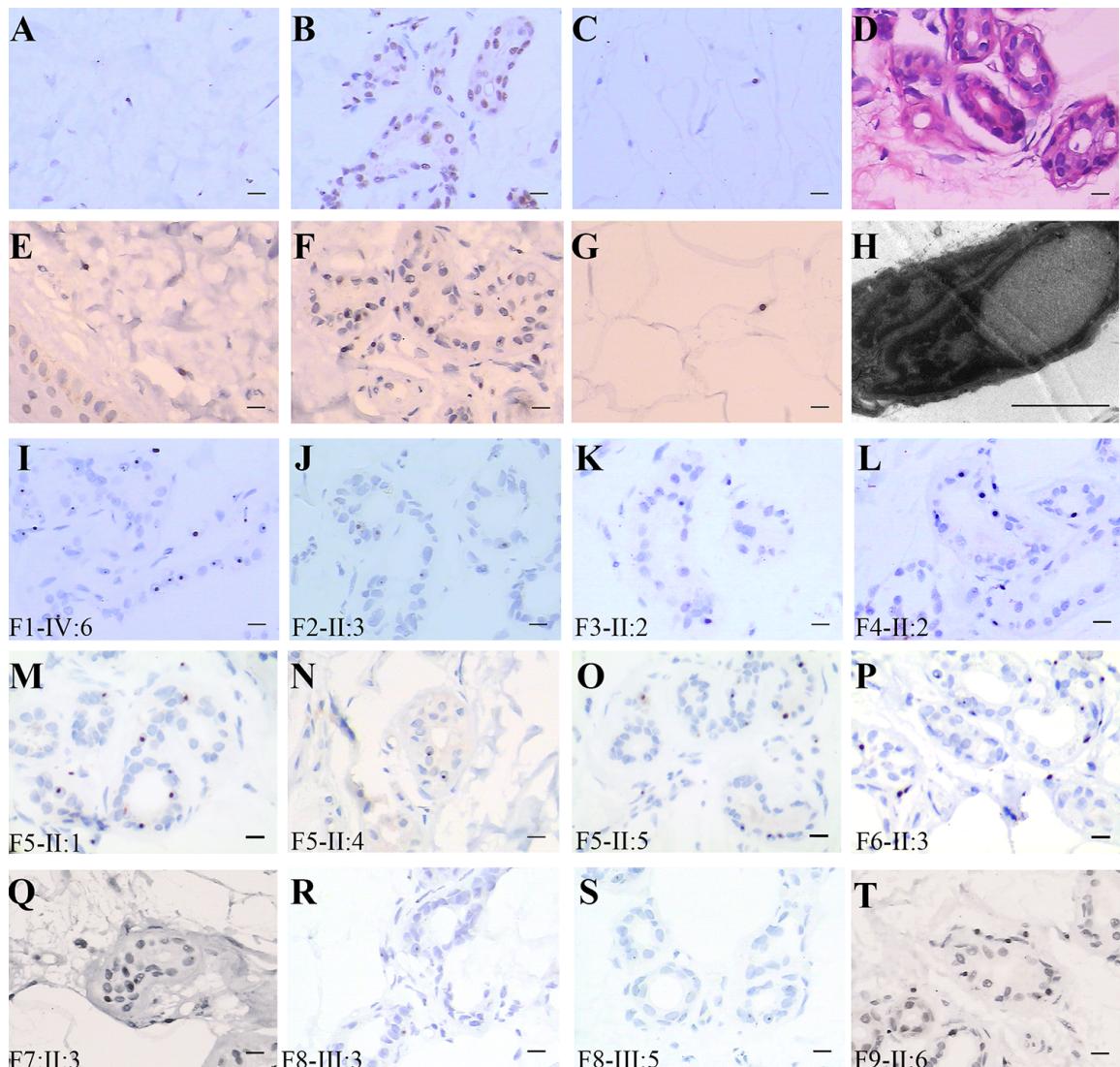


Figure S3. Histopathological Features of NIID. (A-C) Representative skin biopsy samples showing ubiquitin-positive intranuclear inclusions in fibroblasts (A), sweat gland cells (B), and adipocytes (C). Scale bars, 10 μ m. (D) Representative skin biopsy samples revealed eosinophilic intranuclear inclusions in sweat gland cells. Scale bars, 10 μ m. (E-G) Representative skin biopsy samples indicated p62-positive intranuclear inclusions in fibroblasts (E), sweat gland cells (F), and adipocytes (G). Scale bars, 10 μ m. (H) Electron microscopy imaging displaying intranuclear inclusions without membrane. Scale bars, 2 μ m. (I-T) All skin samples showed p62-positive intranuclear inclusions. Scale bars, 10 μ m.

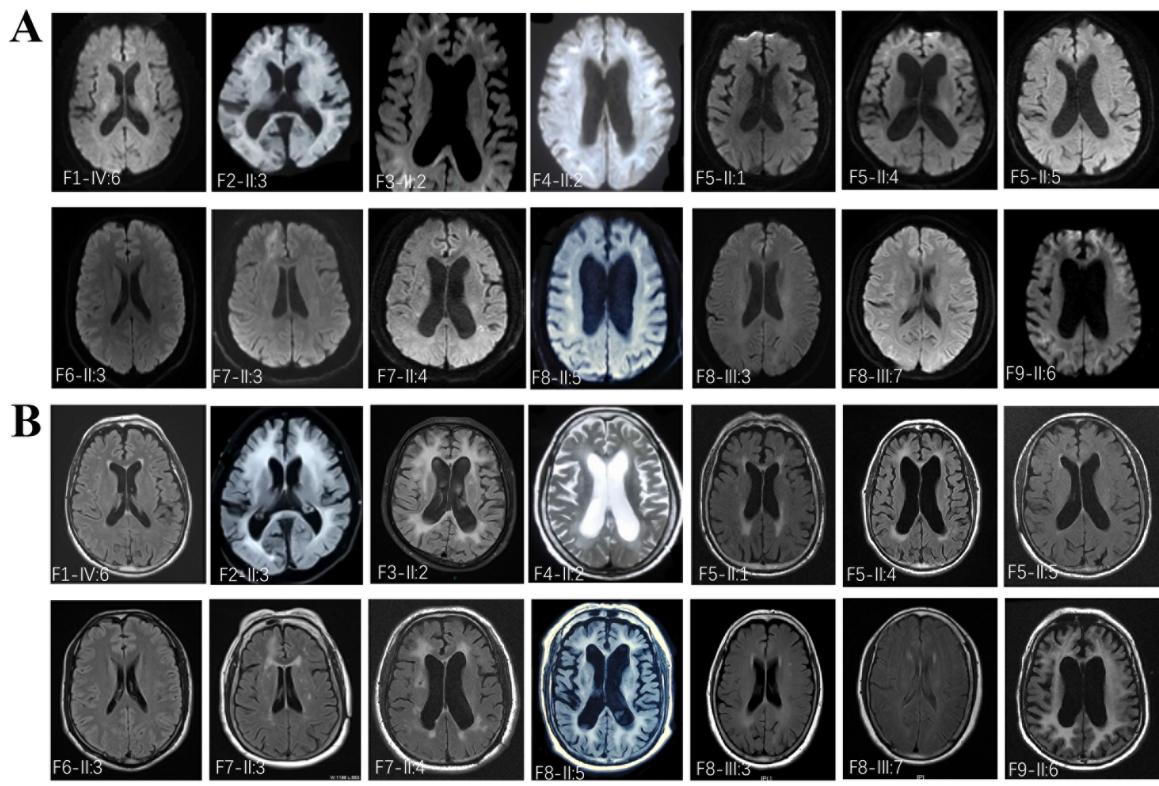


Figure S4. MRI Imaging of NIID. **(A)** DWI images of some NIID-affected case subjects revealed typical symmetrical high signal in corticomedullary junction. **(B)** Several NIID-affected case subjects showed severe white matter hyperintensity using FLAIR or T2 weighted image.

Table S1. Clinical Features of Muscle Weakness-dominant Type (families 1 and 2).

Table S2. Clinical Features of Parkinsonism-dominant Type (families 5-7)

	Family 5			Family 6			Family 7		
	II:1	II:4	II:5	I:1	II:2	II:3	I:1	II:3	II:4
Sex	M	M	M	M	M	M	M	F	M
Age at onset	75	58	66	78	37	46	58	66	61
Disease duration	2	11	2	1	15	3	10	6	4
Clinical manifestations									
Dementia	-	-	-	-	-	-	-	-	-
Abnormal behavior	-	-	-	-	-	-	-	-	-
Peripheral neuropathy									
Muscle weakness	-	+	-	-	-	-	-	-	-
Sensory disturbance	+	-	-	+	-	-	-	+	+
Autonomic dysfunction									
Bladder dysfunction	-	++	-	-	-	+	+++	++	+++
Miosis	-	-	-	-	-	-	/	-	-
Parkinsonism									
Tremor	+	++	-	-	+	-	-	+	+
Rigidity	+	++	+	+	+	+	+	++	+
Bradykinesia	+	++	+	+	+	++	+	++	+
Ataxia	-	+	-	-	-	-	++	+	++
Neurological attack									
Disturbance of consciousness	-	+	-	-	-	-	-	-	-
Stroke-like episode	-	+	+	-	-	-	-	-	-
encephalitic episode	-	-	-	-	-	-	-	-	-
Brain MRI									
Severe leukoencephalopathy	-	+	-	/	/	-	/	-	+
DWI U-fiber high signal	-	+	-	/	/	-	/	-	-
Cognitive function test									
MMSE (<education matched average)	26	27	26	/	29	30	/	22	25
MoCA (<education matched average)	19	15	19	/	/	25	/	/	23
Nerve conduction									
Motor									
MCV slowing	+	+	+	/	/	+	/	++	+
CMAP reduction	+	-	-	/	/	-	/	-	+
Sensory									
SCV slowing	+	-	-	/	/	-	/	++	+
SNAP reduction	-	-	-	/	/	-	/	+	+
Skin biopsy	+	+	+	/	/	+	/	+	/

Table S3. Clinical Features of Dementia-dominant Type (families 3, 4, 8, 9)

	Family 3		Family 4		Family 8						Family 9					
	I:2	II:2	II:3	II:2	II:5	I:1	II:3	II:5	II:8	III:3	III:5	III:7	I:2	II:2	II:3	II:6
Sex	F	M	F	F	F	M	F	M	M	F	F	F	F	M	F	M
Age at onset	71	42	63	49	31	65	60	60	60	58	59	60	60	60	60	71
Disease duration	20	19	2	18	30	5	8	19	16	7	5	3	15	10	12	10
Clinical manifestations																
Dementia	++	-	+	+	++	+++	+++	+++	+++	++	+	-	++	++	++	+++
Abnormal behavior	++	++	+	++	+++	++	++	++	++	-	-	-	++	++	++	+
Peripheral neuropathy																
Muscle weakness	-	-	+	-	-	/	-	+	-	-	-	+	-	-	-	+
Sensory disturbance	+	-	+	-	-	/	-	+	-	-	+	+	-	-	-	-
Autonomic dysfunction																
Bladder dysfunction	+	-	++	++	-	+	+++	+++	+++	-	++	+++	/	+++	+++	+++
Miosis	-	-	-	+	+	/	/	+	/	-	-	-	/	/	/	+
Parkinsonism																
Tremor	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+
Rigidity	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-	+
Bradykinesia	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
Ataxia	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Neurological attack																
Disturbance of consciousness	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+
Stroke-like episode	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
encephalitic episode	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Brain MRI																
Severe leukoencephalopathy	/	/	+	+	/	/	/	+	/	-	-	-	/	/	/	+
DWI U-fiber high signal	/	/	+	+	/	/	/	+	/	-	-	-	/	/	/	+
Cognitive function test							/									
MMSE (<education matched average)	14	29	/	15	12	/	/	/	/	10	26	29	/	/	/	/
MoCA (<education matched average)	/	28	/	/	/	/	/	/	/	3	20	26	/	/	/	/
Nerve conduction																
Motor																
MCV slowing	/	+	/	-	/	/	/	+	/	-	+	+	/	/	/	++
CMAP reduction	/	+	/	-	/	/	/	+	/	-	+	+	/	/	/	++
Sensory																
SCV slowing	/	+	/	-	/	/	/	+	/	-	+	+	/	/	/	+
SNAP reduction	/	+	/	-	/	/	/	+	/	-	+	+	/	/	/	+
Skin biopsy	/	+	/	+	/	/	/	/	/	+	+	+	/	/	/	+

Table S4. Clinical Features of Sporadic NIID-affected Case Subjects

	Sporadic Case Subjects				
	Dementia		Paroxysmal disease		
	SD1	SD2	SP1	SP2	SP3
Sex	F	F	M	M	F
Age at onset	65	69	68	51	57
Disease duration	14	2	1	5	6
Clinical manifestations					
Dementia	++	++	-	-	-
Abnormal behavior	++	+	-	-	-
Peripheral neuropathy					
Muscle weakness	-	-	-	-	-
Sensory disturbance	-	-	-	-	-
Autonomic dysfunction					
Bladder dysfunction	+++	+++	-	++	-
Miosis	+	+	-	-	-
Parkinsonism					
Tremor	+	-	-	-	-
Rigidity	+	-	-	-	-
Bradykinesia	+	-	-	-	-
Ataxia	-	-	-	-	-
Neurological attack					
Disturbance of consciousness	+	+	+	+	-
Stroke-like episode	+	+	+	-	+
encephalitic episode	+	+	-	-	+
Brain MRI					
Severe leukoencephalopathy	+	+	+	+	+
DWI U-fiber high signal	+	+	+	+	+
Cognitive function test					
MMSE (<education matched average)	/	/	27	30	/
MoCA (<education matched average)	/	/	16	29	/
Nerve conduction					
Motor					
MCV slowing	+	/	+	+	/
CMAP reduction	+	/	-	-	/
Sensory					
SCV slowing	+	/	+	+	/
SNAP reduction	+	/	-	-	/
Skin biopsy	+	+	+	+	+