ANNALS of Clinical and Translational Neurology
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AMERICAN NEUROLOGICAL ASSOCIATION

LETTER

Genetic heterogeneity of neuronal intranuclear inclusion disease: What about the infantile variant?

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Funding Information
Ministerstvo Zdravotnictví České Republiky: NV19-08-00137

Received: 25 November 2020; Accepted: 28 January 2021
Annals of Clinical and Translational Neurology **2021; 8(4): 994–1001**

doi: 10.1002/acn3.51332

Dear Editor,

Chen et al.[1] have demonstrated lack of abnormal CGG expansions in the *NOTCH2NLC* gene among European juvenile and adult patients with neuronal intranuclear inclusion disease (jNIID and aNIID). Genetic heterogeneity of NIID was suggested based on the discrepancy of these results and earlier findings in East Asian patients.[2-5]

We recently studied[6] *NOTCH2NLC* CGG repeats in an infantile NIID (iNIID) patient independently of Chen et al.[1] This Caucasian European boy is the first, and thus far only, iNIID patient who has been tested for this molecular pathology. The number of repeats in this patient corresponded to values found in healthy controls. We believe that these data provide further evidence[1,3] for genetic heterogeneity of NIID. It is, however, important to highlight that our results do not, at this point, allow conclusions about genetic causes of NIID among European patients of all age groups or about genetic causes of iNIID.

We recognize the diagnostic criteria for NIID-related diseases that were suggested by Chen et al.[1] However, it is our impression that their algorithm/flowchart considers juvenile and adult NIID patients but the distinctive characteristics of iNIID have not been sufficiently reflected. To help the diagnostic practice, we provide a summary of clinical (Table 1) and (neuro)pathological (Table 2) findings in iNIID patients.

iNIID is a rare and rapidly progressive neurodegenerative pediatric disease.[7,8] To the best of our knowledge, there have been eight ethnically diverse iNIID patients reported.[6,8-14] All were diagnosed by *post-mortem* neuropathology work-up. Seven patients developed the first symptoms prior to or by 5 years of age and died before reaching 10 years of age. Only one patient presented and died older.[9]

The following developmental and clinical characteristics are key phenotypic features of iNIID: (i) early development is more-or-less normal, the onset of disease is abrupt and the progression is rapid; (ii) initial symptoms almost always involve unsteady gait and/or movement abnormalities associated with cerebellar dysfunction, (iii) the terminal neurological disease is associated with cerebellar decline, progressive pseudobulbar or bulbar palsy, peripheral neuropathy, hypotonia, and severe psychosocial regress, and (iv) respiratory infection or respiratory failure-associated pathologies are the most common causes of death. Major non-neurological abnormalities were reported in only one iNIID patient, who suffered from dilated cardiomyopathy and developed congestive heart failure.[13]

Neuroimaging frequently identifies early-onset cerebellar atrophy in iNIID. Contrary to aNIID,[15] there is no information about CNS corticomedullary junction abnormalities and the diagnostic utility of diffusion-weighted MRI in iNIID patients is unknown.

Intranuclear inclusions (IIs) were not found in *ante-mortem* skin biopsies of three iNIID patients.[6,9,10] Skin biopsy of the fourth iNIID patient was reported as normal (presence or absence of IIs was not explicitly noted).[11]

Neuronal IIs are widespread in the central (and also peripheral) nervous system in iNIID patients. IIs in non-neuronal CNS cell types have been unambiguously

Table 1. Clinical history of previously published iNIID patients.

	Sex // ethnicity-nationality // family history	Pre- and perinatal history // early development	Disease onset // initial symptoms	Disease progression	Age at death // cause of death	Brain imaging // electrophysiology
Patel et al. (1985)	**F** // North American Indian// unaffected nonconsaguineous parents	born 1 mth premature (2.2 kg) // developed normal, but free walked at 1.5 yrs; speech delay; poor articulation	**3.5 yrs** // unsteady gait (frequent falls), hypotonia, areflexia, scoliosis, delayed behavioral development, microcephaly (−2SD)	seizures, ataxia, nystagmus, choreoathetosis, cranial nerve palsies, extensor plantar reflexes, poor strength and tone in the arms, sensory deficits in the extremities, diminished pupillary reactions, elevated blood pressure, attacks of bradycardia, and tachycardia 6.5 yrs - developmentally delayed to 4mths	**6 yrs 10mths** // bronchopneumomia, emaciated at autopsy	CT (age *n.r.*) - large ventricles, sulci and cisterns // mild sensory neuropathy (3.5 yrs) severe mixed peripheral neuropathy (6.5 yrs) EEG (age *n.r.*) - diffuse and later bursts of slow activity ECG (age *n.r.*) - normal
Garen et al. (1986)	**M** // *n.r.* // no family history of neurological diseases	uncomplicated pregnancy and delivery // neurologically normal until 5 yrs	**5 yrs** // episodes of loss of motor control in lower extremities (unable to walk), ataxia, transient choreoathetosis	6-7 yrs - increased frequency of motor control loss, progression of dyskinesia, IQ 91 8 yrs - impaired tandem walking, dysmetria, dysarthria, dysphagia, incontinence, blepharospasm 9 yrs - intention myoclonus, conjugate deviation of eyes, episodic nystagmus, cognitive regression, marked tremor and rigidity in the arms, hypotonia of legs, areflexia, immobile, unable to chew or swallow, gastrostomy	**9.5 yrs** // pneumonia	CT (9 yrs) – unspecified mild atrophic changes // VERs and BAERs (age *n.r.*) - mildly abnormal slow conduction in peroneal nerves (age *n.r.*) EEG (9 yrs) - slow waves
Oyer et al. (1991)	**M** // white // *n.r.*	born premature (36wks) // normal development until 3 yrs	**3 yrs** // incoordination and disturbed gait	3-6 yrs - loss of speech and head control, ptosis, athetosis, drooling 8 yrs - congestive heart failure and pneumonia	**9 yrs** // *n.r.*	MRI (6 yrs) - atrophy of cerebral cortex, cerebellum, cerebral peduncles, and olives

(*Continued*)

Table 1. Continued.

	Sex // ethnicity-nationality // family history	Pre- and perinatal history // early development	Disease onset // initial symptoms	Disease progression	Age at death // cause of death	Brain imaging // electrophysiology
				9 yrs - bedridden, progressive neurologic deterioration, terminal diagnosis – variant olivopontocerebellar atrophy syndrome		chest X-ray and echocardiography (8 yrs) - cardiomegaly, pulmonary edema, ventricular dysfunction, small pericardial effusion // *n.r.*
Sloane et al. (1994)	**M** // *n.r.* // healthy nonconsaguineous parents and older brother, maternal uncle and grandmother – Parkinson disease, maternal first cousin – intellectual disability of unknown cause	uncomplicated pregnancy and delivery, born at term (3460 g) // motor development normal until 3 yrs	**2.5 yrs** // ataxia, speech delay, seizures	3 yrs 1 mth - delay in all areas of Yale Developmental Schedule 3.5 yrs - progressive ataxia (wheelchair bound), resting tremor, titubation, incontinence, speech deterioration, loss of communication skills, myoclonic and absence seizures refractory to therapy 4.5 yrs - little verbal and non-verbal communication, nystagmus, bilateral internuclear ophtalmoplegia, generalized hypotonia, mild pes cavus, marked truncal and appendicular ataxia	**4 yrs 4 mths** // found unconscious at home, acute bronchitis and pulmonary edema at autopsy	CT (3.5 yrs) - cerebellar atrophy MRI (consequent to CT) - vestigal vermis and superior vermal atrophy, small brainstem, dilated aqueduct, 4th ventricle and CSF spaces // EEG (2.5 yrs) - generalized spike-and-wave activity, later generalized slow waves BAERs (age *n.r.*) - hearing loss, poor delineation of wave 1 but normal central conduction VERs, somatosensory EPs in median nerve, and peripheral motor nerve conduction (age *n.r.* in all) - normal.
McFadden (2005)	**M** // *n.r.* // no family history of dementia, developmental or movement disorders, motor handicaps or seizures	uncomplicated pregnancy and delivery // normal early developmental milestones	**7.5 yrs** // tremors of hands, difficulty walking, required assistance after falling, leg pain and weakness, stiff-legged	8 yrs - action and sustension tremor, bilateral weakness of lower legs, decreased deep tendon reflexes, pes planus, impaired speech articulation	**13 yrs** // respiratory infection and failure, emaciated and with generalized muscle atrophy at autopsy	MRI (8 yrs + 9.5 yrs) - mild enlargement of cerebellar sulci and mild vermian atrophy // reduced motor and sensory conduction velocities in both upper

(*Continued*)

Table 1. Continued.

	Sex // ethnicity-nationality // family history	Pre- and perinatal history // early development	Disease onset // initial symptoms	Disease progression	Age at death // cause of death	Brain imaging // electrophysiology
			gait, right leg rotated outwards	9 yrs - wheelchair bound, no speech, difficulty swallowing, excessive drooling, coarse tremor with episodes of whole body shaking, positive extensor plantar reflexes, oculogyric crises 11 yrs - constant tremor of hands, unable to hold head erect, drooling (atrophic tongue with fasciculations), generalized muscular atrophy, contractures and areflexia		and lower extremities (8 yrs) sural sensory action potential (8 yrs) - unobtainable electromyography (8 yrs) – denervation/ reinnervation changes without evidence of recent denervation ECG (8 yrs) - normal
Mano et al. (2007)	**M** // Japanese // no family history of neurological illnesses	normal pregnancy and delivery, born at 40 wks (2520 g), neonatal period uneventful // psychomotor development normal until 2 yrs	**2 yrs** // afraid of walking	4 yrs – progressive deterioration, open mouth, hypersalivation, generalized hypotonia, spasticity in lower limbs with hyperactive deep tendon reflexes, slurred speech, ataxia, unable to stand or walk without support 5-7 yrs - worsened bulbar palsy, unable to sit unsupported, seldom speaking, dysphagia, intellectual deterioration, gavage feeding, tracheostomy, bedridden	**7 yrs** // respiratory failure, hypoventilation, pneumonia	MRI (2 yrs) - marked cerebellar atrophy; MRI (4 yrs) - severe atrophy of vermis and cerebellar hemispheres, mild brainstem atrophy, no cerebral atrophy, no myelination abnormality; SPECT (4 yrs) - cerebellar hypoperfusion; MRI (5-7 yrs) - severe cerebellar and cerebral (grey and also little in white matter) atrophy // EEG (4 yrs) slow basic rhythm, no paroxysmal activity; normal peripheral nerve conductance (4 yrs),

(*Continued*)

Table 1. Continued.

	Sex // ethnicity-nationality // family history	Pre- and perinatal history // early development	Disease onset // initial symptoms	Disease progression	Age at death // cause of death	Brain imaging // electrophysiology
						EEG (5-7 years) - continuous diffuse sharp and slow waves, no convulsions
Pilson et al. (2017)	**F** // African // *n.r.*	*n.r.* // appeared to develop normally until 4 yrs	**4 yrs** // found convulsing and febrile, seizures since then, dyskinesia, unable to walk or sit unsupported	5.5 yrs – developmental regression, increased seizure activity, poor central tone, reduced global power, loss of pincer grasp, limited vocabulary, nystagmus	**6.5 yrs** // aspiration pneumonia, upper and lower limb wasting at autopsy	MRI (5.5 yrs) - dilatation of the 4th ventricle, significant cerebellar atrophy
Jedlickova et al. (2020)	**M** // Caucasian-Czech // no family history of neurological illnesses, healthy parents and older sister	born at term, spontaneously, adapted normally // psychosocial and motor development normal until 2 yrs, verbal development delayed at 2 yrs	**2 yrs 4 mths** // intention tremor, ataxia, convergent strabismus	2 yrs 8 mths – rapidly progressive cerebellar ataxia **terminal neurologic disease** - severe progressive neurodegenerative condition with psychosocial and cognitive regression, pseudobulbar syndrome, mixed axonal and demyelinating polyneuropathy	**7 yrs** // chronic respiratory failure with terminal bronchopneumonia	MRI (2 yrs 4 mths) - cerebellar atrophy MRI (2 yrs 8 mths) - progressed cerebellar atrophy // EEG (2 yrs 4 mths) - slower basal activity, no epileptic activity sleep EEG (2 yrs 8 mths) - bilateral epileptiform discharges (no symptomatic seizures)

Abbreviations: BAERs, brain stem auditory-evoked responses; CT, computer tomography; ECG, electrocardiography; EEG, electroencephalography; EP, evoked potentials; F - female; M, male; MRI, magnetic resonance imaging; mths, months; *n.r.*, not reported; SD, standard deviation; SPECT, single-photon emission CT; VERs, visual-evoked responses; wks, weeks; yrs, years.

Table 2. Biopsy and autopsy findings in iNIID patients.

	Ante-mortem biopsies	Brain weight (g) // gross CNS pathology	CNS histopathology	Ultrastructure of the intranuclear inclusions	Pathology of non-CNS tissues
Patel et al. (1985)	**skin bs (age *n.r.*) - normal** skeletal muscle bs (age *n.r.*) – normal peripheral (sural) nerve bs (age *n.r.*) - increased endoneural collagen and some loss of unmyelinated axons	900 // mild frontal gyral atrophy, slightly enlarged left lateral ventricle, small semioval center, cerebellar vermal atrophy	widespread neuronal IIs neurodegeneration - PCs, granule cells layer neurons, thalamus, DN mild myelin loss in the mid-portions of the posterior (spinal) columns in the mid-thoracic area and caudad	ranging from grey fluffy masses to filamentous	DRG neurons, autonomic ggl., myenteric plexi neurons - IIs mild myelin pallor of the lumbal dorsal roots cells of the adrenal medulla and anterior pituitary gland - IIs
Garen et al. (1986)	*n.r.*	1160 // no atrophy of the brain; gray and white matter grossly normal; pale SN; no atrophy of SC	widespread IIs confined to neurons neurodegeneration - pigmented SN neurons, Clarke's column neurons, PCs with hypoxic/ischemic alterations mild loss of myelin in fasciculus gracilis, ventral and dorsal spinocerebellar tracts	filamentous with dense core (some granularity at high magnification)	DRG neurons - IIs
Oyer et al. (1991)	*n.r.*	1119 // olivary bulges slightly atrophic; subthalamic nuclei difficult to distinguish; firm pyramidal tracts and olivary nuclei; atrophy of the cerebellar folia	widespread neuronal IIs	granular (in cardiomyocytes)	DRG neurons - IIs dilated cardiomyopathy with fibrosis (heart weight - 273g) cardiomyocytes - IIs skeletal muscle with atrophy but no IIs
Sloane et al. (1994)	*n.r.*	590 // normal cerebral hemispheres, brain stem and spinal cord; small cerebellum with atrophy of hemispheres and vermis; dilated 4th ventricle	widespread neuronal IIs; severe cerebellar ctx atrophy neurodegeneration - PCs, granule cells layer neurons, and DN	filamentous	DRG neurons, sympathetic ggl. and myenteric plexi neurons – IIs hepatocytes – IIs skeletal muscle (deltoid and quadriceps) - no abnormality
McFadden (2005)	sural nerve bs (8 yrs) - axonal degeneration and regeneration **skin biopsy (8 yrs) - no IIs in any cell type**	1425 // atrophy of the cerebellar hemisphere and vermis (hemidisected brain), mild atrophy of the SC ventral roots	widespread neuronal IIs, glial IIs not seen neurodegeneration - subthalamic nucleus, SN, red ncl., cerebellar ctx (severe PC loss, moderate granular cells loss), DN, SC anterior horn cells IHC - IIs SUMO-1+, ubiquitin+, glucocorticoid receptor+ deep white matter well myelinated	randomly oriented filaments and electron-dense granular material	neurons of the adrenal medulla and Auerbach's plexus – IIs cardiac cells – occasional IIs

(*Continued*)

Table 2. Continued.

	Ante-mortem biopsies	Brain weight (g) // gross CNS pathology	CNS histopathology	Ultrastructure of the intranuclear inclusions	Pathology of non-CNS tissues
Mano et al. (2007)	superficial rectal bs - not conclusive	1080 // cerebellar and brainstem atrophy	widespread neuronal IIs, <5% of astrocytes with IIs, oligodendrocytes, ependymal and endothelial cells with no IIs marked neurodegeneratrion of the cerebellar ctx and inferior olive, moderate to mild neurodegeneration in DN and pontine nuclei, mild degeneration in cerebral ctx, striatum and ventral SC horns myelin pallor due axonal loss in the cerebral hemisphere, cerebral peduncles, medullary pyramid and SC corticospinal tract IHC - IIs ubiquitin+	microfibrillary	DRG - IIs, Meissner and Auerbach plexus – occasional IIs
Pilson et al. (2017)	**skin bs (collected 7mths prior death) - no IIs reported** muscle bs (age *n.r.*) - neurogenic atrophy	*n.r.* // normal cortical mantle, atrophic cerebellar vermis, dilated 4th ventricle	widespread neuronal IIs cerebellar atrophy with PC loss and Bergmann gliosis IHC - IIs p62+, ubiquitin+	*n.r.*	myenteric plexus of the esophagus, stomach, appendix, and small and large intestines, parasympathetic ganglia and neural crest cells of the adrenal medulla - IIs
Jedlickova et al. (2020)	**skin bs (2 yrs 8 mths) - no IIs**	1220 // normal cerebral globation and gyrification, atrophy of cerebellar vermis and hemispheres	widespread and almost exclusively neuronal IIs widespread neurodegeneration, severe cerebellar atrophy with PC loss and astrocytosis IHC - IIs p62+, ubiquitin+	fibrillary	lungs, spleen, kidneys, liver and myocardium – no IIs

Abbreviations: bs, biopsy; CNS, central nervous system; ctx, cortex; DN, dentate nucleus; DRG, dorsal root ganglia; ggl, ganglia; IHC, immunohistochemistry; IIs, intranuclear inclusions; mths, months; *n.r.*, not reported; ncl, nucleus; PCs, Purkinje cells; SC, spinal cord; SN, substantia nigra; yrs, years.

reported in only one patient (less than 5% of astrocytes contained IIs).[8] The extent and distribution of CNS neuronal loss may be variable, however, cerebellar (cortical) atrophy is almost universally present in iNIID patients. Mild myelin loss and/or pallor in different CNS regions was noted only occasionally.

To summarize: iNIID is a severe early childhood-onset neuropediatric disease. Some of the key clinical, neuroimaging and (neuro)pathology characteristics that allow *ante-mortem* identification of jNIID and aNIID may not be useful to diagnose iNIID patients. Understanding the genetic background of iNIID is a critical component to allow effective diagnostics, counselling and future therapy development. With this information at hand, we are prepared to collaboratively study our data with samples from other children with iNIID or within cohorts of juvenile and adult NIID individuals.

Acknowledgments

This work was supported by the research grant (NV19-08-00137) from the Ministry of Health of the Czech Republic.

Conflict of Interest

All authors report no disclosures.

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