Neuronal Intranuclear Inclusions in Spinocerebellar Ataxia Type 2

Shigeru Koyano,*† Toshiki Uchihara,† Hiroto Fujigasaki,†‡
Ayako Nakamura,† Saburo Yagishita,§
and Kiyoshi Iwabuchi*

The inherited neurodegenerative disorders such as the spinocerebellar ataxias, Huntington's disease, dentatorubral-pallidoluysian atrophy, and spinal and bulbar muscular atrophy associated with CAG/polyglutamine repeat expansion have been reported to exhibit intranuclear inclusions (NIs) in neurons.[1] In *Annals*, Huynh and colleagues[2] have reported the regional distribution of the spinocerebellar ataxia type 2 (SCA2) gene product, ataxin-2, in SCA2 brains. Although they identified ataxin-2-like immunoreactivity in the cytoplasm of neurons, NIs were reported to be absent in the cerebellum of SCA2 brains in their study.

We performed an immunohistochemical study to identify the immunolocalization of ataxin-2 in the cerebellum and other brain regions of autopsy brains from 3 Japanese patients genetically confirmed as having SCA2. We used antibodies against ataxin-2 (a well-characterized monoclonal antibody),[3] expanded polyglutamine (mAb 1C2),[4] and ubiquitin. Formalin-fixed, paraffin-embedded sections were stained by a standard immunohistochemical technique after pretreatment with periodic acid and microwave oven. This ataxin-2-like immunoreactivity was mainly in the cytoplasm and, to a lesser extent, in the nuclei of neurons, and NIs were not found in Purkinje cells in any of these three SCA2 cases, as reported by Huynh and colleagues.[2] In addition, we identified a round intranuclear structure immunolabeled by three antibodies (Fig) outside the cerebellum. They were usually solitary, distinguishable from the nucleolus and 1 to 5 mm in diameter. Because they were not found in nonneuronal cells or in normal brains, they were assumed to correspond to NIs. In all these three SCA2 brains, NIs were found most frequently in the ventral region of the pons, and 1% to 2% of the remaining pontine neurons contained NIs. They appeared in affected (the pontine nuclei, substantia nigra, and inferior olivary nucleus) but not in unaffected regions (the dentate nucleus and cranial nerve nuclei). Identification of NIs in SCA2 brains indicates that SCA2 may, at least partly, share pathogenic mechanisms common to other CAG/polyglutamine repeat disorders with NIs.

**Department of Neurology and Psychiatry and §Department of Pathology, Kanagawa Rehabilitation Center, Atsugi, Japan; and †Department of Neuropathology, Tokyo Metropolitan Institute for Neuroscience, and ‡Department of Neurology, Tokyo Metropolitan Neurological Hospital, Tokyo, Japan*

References

1. Davies SW, Beardsall K, Turmaine M, et al. Are neuronal intranuclear inclusions the common neuropathology of triplet-repeat disorders with polyglutamine-repeat expansions? Lancet 1998;351:131–133
2. Huynh DP, Del Bigio MR, Ho DH, Pulst SM. Expression of ataxin-2 in brains from normal individuals and patients with Alzheimer's disease and spinocerebellar ataxia 2. Ann Neurol 1999; 45:232–241
3. Lettink M, Lewis JG, Elder PA. A monoclonal antibody raised against amino acids 497–507 of the human estrogen receptor recognizes human ataxin-2. J Biochem Mol Biol Biophys 1999; 2:111–119
4. Trottier Y, Lutz Y, Stevanin G, et al. Polyglutamine, expansion as a pathologic epitope in Huntington's disease and four dominant ataxias. Nature 1995;378:403–406

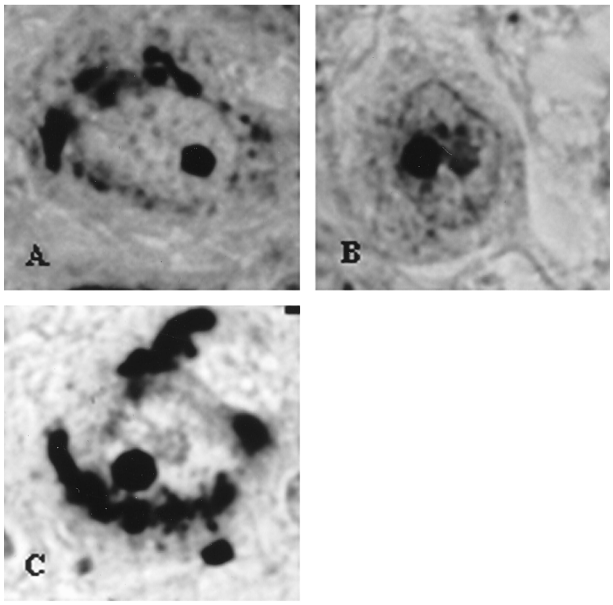

Fig. Intranuclear inclusions in pontine nuclei of patient with spinocerebellar ataxia type 2 immunostained by anti-ataxin-2 (A), anti-ubiquitin (B), and 1C2 (C). Magnification, ×1,000 (A–C).

Valproate for Girls with Epilepsy

Adam H. Balen, MD, MRCOG,*
and Pierre Genton, MD†

We read with interest the article by Vainionpää and colleagues,[1] which is a further account from the same Finnish group on the putative association between the use of valproate and the development of polycystic ovary syndrome (PCOS).[2]

The pathogenesis of PCOS seems to be multifactorial, with both genetic and environmental influences.[3] Hyperinsulinemia, or a genetic predisposition to hyperinsulinemia, now seems to be a key factor. Other potential factors in the pathogenesis of PCOS include genetic abnormalities of steroidogenesis, disturbances of the regulation of the hypothalamus, and genetic variants of luteinizing hormone.[3] It is known that menstrual cycle disturbances are common in young women, with up to 33% having polycystic ovaries,[4] and fewer, perhaps 5% to 10%, having symptomatic PCOS. It has also been reported that menstrual cycle disturbances are common in women with epilepsy, and, in addition, the type of epilepsy may have a further effect. Changes in weight effect the expression of PCOS in individuals with polycystic ovaries, with a gain in weight worsening both symptomatology and metabolic and endocrine profiles. Valproate may in-

duce weight gain in some individuals, and these may be at risk for clinically significant hormonal changes.

This latest article provides interesting, yet inconclusive, data on the real association between the use of valproate and the genesis of PCOS. Unfortunately, yet again, there is no baseline data to indicate body mass index, ovarian morphology, or serum endocrinology before starting treatment. In our view, it is not sufficient to compare young patients with epilepsy on therapy with a control population. It must be noted that there are many causes for epilepsy in this group of patients, some of which may be associated with obesity. The only way that we will know whether valproate induces PCOS is to prospectively randomize patients with newly diagnosed epilepsy to receive either valproate or an alternative antiepileptic drug. We are in the process of coordinating such a multicenter study in the United Kingdom. Until the relationship between the use of valproate and the occurrence of PCOS is firmly established on such a basis, we see no reason to refrain from using valproate in girls and young women, although we recommend strict monitoring of weight in these patients.

**Leeds General Infirmary, Leeds, UK, and †Centre Saint Paul, Marseille, France*

References

1. Vainionpää LK, Rättyä J, Knip M, et al. Valproate-induced hyperandrogenism during pubertal maturation in girls with epilepsy. Ann Neurol 1999;45:444–450
2. Isojärvi JIT, Laatikainen TJ, Pakarinen AJ, et al. Polycystic ovaries and hyperandrogenism in women taking valproate for epilepsy. N Engl J Med 1993;329:1383–1388
3. Balen AH. Pathogenesis of polycystic ovary syndrome—the enigma unravels? Lancet 1999;18:966–967
4. Michelmore KF, Balen AH, Dunger DB, Vessey MP. Polycystic ovaries and associated clinical and biochemical features in young women. Clin Endocrinol 1999;51:779–786

Reply

Jouko I. T. Isojärvi, MD, PhD,*
Leena K. Vainionpää, MD, PhD,†
Mikael Knip, MD, PhD,‡
and Juha S. Tapanainen, MD, PhD§

In their letter to the editor, Balen and Genton comment on our recently published article on reproductive endocrine findings in girls taking valproate for epilepsy.[1] The authors emphasize the importance of weight gain and hyperinsulinemia in the pathogenesis of reproductive endocrine disturbances related to the use of valproate. They also stress strict monitoring of weight gain to find/prevent these hormonal changes. However, in the study of Vainionpää and colleagues,[1] the most important finding was that hyperandrogenism was observed in 38% of prepubertal, 36% of pubertal, and 57% of postpubertal girls taking valproate, and that hyperandrogenism was not associated with obesity or hyperinsulinemia in these girls. Thus, it is not appropriate to emphasize weight gain as a sole factor leading to reproductive endocrine disorders in valproate-treated girls with epilepsy.

In our studies in adult women with epilepsy, we have seen polycystic ovaries, hyperandrogenism, or both in approximately 70% of women taking valproate monotherapy for epilepsy. However, the occurrence rate has been close to 20% in women taking carbamazepine, which is similar to the frequency seen in control women.[2,3] In our studies, most women with polycystic ovaries or hyperandrogenism during valproate medication have had menstrual disorders.[2,3] These disorders are more frequent in obese valproate-treated women with hyperinsulinemia, but they are also seen in lean women without overt hyperinsulinemia.[3]

It is true that menstrual cycle disturbances are common in women with epilepsy and that the type of epilepsy may have a contributing effect. However, in our patients, the frequency of polycystic ovaries and hyperandrogenism has been high in valproate-treated women regardless of whether they have had partial or primary generalized epilepsy.[2,3] Replacing valproate medication with lamotrigine resulted in restoration of normal serum testosterone and insulin levels in women with polycystic ovaries and hyperandrogenism detected during valproate medication. Ovarian structure and menstrual cycles became normal in most of these women during the first year after valproate was tapered off. There was no significant change in seizure control among these women, which suggests that the endocrine changes were associated with valproate medication rather than epilepsy per se.[4] Moreover, it has been recently shown that valproate induces changes in ovarian structure and serum sex steroid concentrations in female rats without epilepsy.[5]

We agree with Balen and Genton[1] that strict monitoring of weight is important during valproate medication. However, the reproductive endocrine changes related to valproate medication cannot be revealed merely by monitoring body weight. We feel that monitoring menstrual cycle length and serum testosterone levels in girls and women taking valproate medication is important in this respect. Ovarian ultrasonography is useful in assessing ovarian structure. A prospective randomized study on the endocrine effects of valproate is important in evaluating the relationship between the use of valproate and the occurrence of polycystic ovaries and hyperandrogenism in women with epilepsy. However, this would involve a long-term study, because it may take years for these changes to emerge. Meanwhile, we feel that our female patients currently taking valproate are entitled to know whether there are changes in their ovarian structure and function. If changes have occurred, an alternate medication should be considered.

*Departments of *Neurology, †Pediatrics, and §Obstetrics and Gynaecology, University of Oulu, Oulu; and ‡Department of Pediatrics, Medical School, University of Tampere, Tampere, Finland*

References

1. Vainionpää LK, Rättyä J, Knip M, et al. Valproate-induced hyperandrogenism during pubertal maturation in girls with epilepsy. Ann Neurol 1999;44:444–450
2. Isojärvi JIT, Laatikainen TJ, Pakarinen AJ, et al. Polycystic ova-

ries and hyperandrogenism in women taking valproate for epilepsy. N Engl J Med 1993;329:1383–1388
3. Isojärvi JIT, Taubøll E, Pakarinen AJ, et al. Valproate related endocrine risks in women with epilepsy. Epilepsia 1998;39(Suppl 6):S220–S221
4. Isojärvi JIT, Rättyä J, Myllylä VV, et al. Valproate lamotrigine, and insulin-mediated risks in women with epilepsy. Ann Neurol 1998;43:446–451
5. Taubøll E, Isojärvi JIT, Flinstad Harbo H, et al. Effects of long-term valproate treatment on serum sex steroid hormone levels and ovarian morphology in female wistar rats. Seizure 1999;8: 490–493

Simvastatin and Plasma Very-Long-Chain Fatty Acids in X-Linked Adrenoleukodystrophy

Aad Verrips, MD,* Michèl A. A. P. Willemsen, MD,* Estela Rubio-Gozalbo, MD,† Jan De Jong, PhD,‡ and Jan A. M. Smeitink, MD, PhD†‡

X-linked adrenoleukodystrophy (X-ALD) is a peroxisomal disorder, in which ongoing accumulation of very-long-chain fatty acids (VLCFAs) in the central nervous system, adrenal cortex, and testes, together with secondary inflammatory damage, can lead to four phenotypes, ie, childhood cerebral, adrenomyeloneuropathy, Addison-only, or an asymptomatic form.[1,2] Several treatment regimens have been used in X-ALD patients without clinical benefit.[1] Lovastatin, a 3-hydroxy-3-methylglutaryl–coenzyme A reductase (HMGCoA-R) inhibitor, can inhibit the inducible nitric oxide synthase and proinflammatory cytokines in rat astrocytes, microglia, and macrophages. These cells are thought to be involved in the pathogenesis of neurological damage in X-ALD.[3] Addition of lovastatin to X-ALD skin fibroblasts normalizes VLCFA concentrations in these cells.[4] Seven adult X-ALD patients, treated with lovastatin for 2 to 6 months, achieved normal plasma levels of total VLCFAs.[5]

We evaluated the efficacy and tolerability of increasing doses of simvastatin, 0.2 to 1.0 mg/kg of body weight/day, during an open-label study on 6 children with X-ALD over a period of 3 months. Informed consent from the parents and the patients was obtained. Lorenzo's oil treatment had been withdrawn at least 1 month earlier and diet was normal. Clinical assessment was performed at 2-week intervals. Plasma VLCFAs, cholesterol, liver and renal function, and creatine kinase were evaluated once a month.

All 6 children completed the trial. One child showed progressive disease; the others remained stable. Excessive flatulence during the maximum doses of simvastatin was present in 2 children. Routine laboratory safety tests remained normal. Plasma total cholesterol concentration decreased by 22%. No significant decrease was seen in plasma VLCFA concentrations (Table). Our findings contrast with those of Singh and colleagues.[5] These results might be due to the use of simvastatin instead of lovastatin, independent of the HMGCoA-R inhibition effect. It has not yet been proved in vivo whether statins stimulate VLCFA β-oxidation by inhibiting inducible nitric oxide synthase and cytokine release. This differs from direct enzyme inhibition of HMGCoA-R, which results in down-regulation of cholesterol synthesis.

Moreover, in children, stimulation of VLCFA β-oxidation might depend on higher or continuously high plasma statin levels. This hypothesis implicates a possible effect of higher doses or smaller time intervals between doses. Preferably, more pilot studies with different statins and dosage schedules should be performed before large, multicenter, placebo-controlled clinical trials can be conducted.

*Departments of *Paediatric Neurology, †Paediatrics, and ‡Laboratory of Neurology and Paediatrics, University Hospital Nijmegen, Nijmegen, The Netherlands*

References

1. Moser HW. Adrenoleukodystrophy: phenotype, genetics, pathogenesis and therapy. Brain 1997;120:1485–1508
2. Dubois Dalcq M, Feigenbaum V, Aubourg P. The neurobiology of X-linked adrenoleukodystrophy, a demyelinating peroxisomal disorder. Trends Neurosci 1999;22:4–12
3. Pahan K, Sheikh FG, Namboodiri AM, Singh I. Lovastatin and phenylacetate inhibit the induction of nitric oxide synthase and cytokines in rat primary astrocytes, microglia, and macrophages. J Clin Invest 1997;100:2671–2679

Table. Mean (and SD) Plasma Levels of VLCFAs and Cholesterol in 4 Children with Childhood Cerebral X-Linked Advenoleukodystrophy (Age, 7–10 Years) and 2 Children with Addison-Only X-Linked Adrenoleukodystrophy (Age, 3 and 8 Years) at Diagnosis, Before Entering the Study, and During Simvastatin Therapy

	Reference Range	Diagnosis	$t = 0$ (Before Treatment)	$t = 1$ Mo, 0.2–0.5[a]	$t = 2$ Mo, 1[a]	$t = 3$ Mo, 1[a]
VLCFAs						
C24:0/C22:0	0.72–1.02	1.76 (0.076)	1.75 (0.168)	1.87 (0.219)	1.81 (0.155)	1.74 (0.164)
C26:0/C22:0	0.008–0.026	0.073 (0.009)	0.067 (0.019)	0.071 (0.019)	0.073 (0.012)	0.065 (0.007)
C26:0 (μM)	0.51–1.05	2.78 (0.256)	2.47 (0.589)	2.17 (0.427)	2.25 (0.624)	2.35 (0.540)
Total cholesterol (mmol/L)	2.6–5.2		3.7 (0.651)			2.9 (0.304)
LDL cholesterol (mmol/L)	Up to 4.7		2.1 (0.892)			1.4 (0.232)
HDL cholesterol (mmol/L)	0.95–1.50		1.0 (0.206)			1.2 (0.196)

[a]mg of simvastatin/kg of body weight/day.

VLCFA = very-long-chain fatty acid; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

4. Singh I, Pahan K, Khan M. Lovastatin and sodium phenylacetate normalize the levels of very long chain fatty acids in skin fibroblasts of X-adrenoleukodystrophy. FEBS Lett 1998;426:342–346
5. Singh I, Khan M, Key L, Pai S. Lovastatin for X-linked adrenoleukodystrophy. N Engl J Med 1998;339:702–703

Mirror Agnosia: The Ramachandran Sign

Eric Lewin Altschuler, MD, PhD

In May 1997, we published an article "Mirror Agnosia"[1] in which we noted that we had presented preliminary results in an abstract in 1996. Patients with parietal strokes were instructed to turn their head to the right and look at a mirror being held by one investigator. Another investigator standing on the left side of the patient held a pen. The patients were then asked to "reach out and grab the pen."[1] Remarkably, the patients "reach[ed] without hesitation straight into the mirror and kept banging repeatedly into the mirror,"[1] although the patients were otherwise mentally lucid, could see clearly, and were aware it was a mirror into which they were banging, and could describe the properties of a mirror. We called this new neurological sign "mirror agnosia," because the patients see the mirror clearly but behave as if it does not exist. We proposed that mirror agnosia might be either (1) "a specific consequence of neglect," or (2) "instead, . . . a striking manifestation of the subtle deficits in spatial abilities that occur following parietal lobe lesions."[1] Our study was quickly cited by others.[2–4]

We were pleased to see our work replicated by one of the finest neuroimaging groups in the world, Binkofski and colleagues.[5] But, we were surprised to see that their abstract described mirror agnosia as a "new" syndrome. This is not the case. By all internationally accepted conventions for scientific nomenclature, our priority is clear.[1]

Curiously, these German and Italian investigators use nearly the same American colloquialism, in instructions to patients, as we did, ie, "reach out and grab the object."[5] They incorrectly state that we had said that mirror agnosia may be "caused by a disturbance of spatial abilities after right parietal lobe damage." They also claim to have found a new syndrome called "mirror ataxia." However, because many of their patients with mirror ataxia had, in fact, mirror agnosia,[1] further work will be necessary to show that mirror ataxia is an independent syndrome that can be doubly disassociated from mirror agnosia[1] and/or the well-known problems that patients with parietal lesions can have with spatial and reaching tasks. Henceforth, mirror agnosia should be known as the "Ramachandran sign."

Brain and Perception Laboratory, University of California, San Diego, La Jolla, CA

References

1. Ramachandran VS, Altschuler EL, Hillyer S. Mirror agnosia. Proc R Soc Lond B 1997;264:645–647
2. Turnbull OH. Neglect: mirror, mirror, on the wall—is the left side there at all? Curr Biol 1997;7:R709–R711
3. Goebel R, Linden DE, Lanfermann H, et al. Functional imaging of mirror and inverse reading reveals separate coactivated networks for oculomotion and spatial transformations. Neuroreport 1998;9:713–719
4. Cantagallo A, Della Sala S. Preserved insight in an artist with extrapersonal spatial neglect. Cortex 1998;34:163–189
5. Binkofski F, Buccino G, Dohle C, et al. Mirror agnosia and mirror ataxia constitute different parietal lobe disorders. Ann Neurol 1999;46:51–61

Reply

Ferdinand Binkofski, Rüdiger J. Seitz, and Hans-Joachim Freund

At the same time and in different parts of the world, it was observed that patients with parietal strokes may suffer from the inability to reach to an object presented to them through a mirror. In 1997 Ramachandran and colleagues described 4 such patients, all of them with left-sided visual hemineglect following right hemispheric stroke involving the parietal lobe.[1] When objects in the left neglected hemifield were shown to them through a mirror placed in the sagittal plane on their right side, they thought the object would be in the mirror and pointed there with their right, nonparalyzed arm. The authors coined the term *mirror agnosia* for this phenomenon and discussed neglect or a spatial parietal lobe disability as two possible explanations.

In our published abstract,[2] which had been submitted before the publication of the article by Ramachandran and colleagues,[1] we reported on 5 patients with parietal lobe lesions of either side who also mistook the mirror for the object and reached to the virtual object in the mirror rather than towards the real object. Except for this common denominator in their and in our patients everything else was different: our patients had no neglect, made the mirror-related error in all directions and with both arms, had their lesions in either parietal lobe, and performed poorly in other spatial functions, such as the line orientation and the mental rotation test and the adjustment of the subjective body vertical. In a subsequent thorough examination of these and additional patients,[3] we extended our initial observations by neuropsychological and kinematic data and by lesion analysis. In addition, we described another group of patients that could recognize the mirror and knew that the object was somewhere else.[3] Although they could flawlessly point to objects directly, in the mirror condition, their movements became grossly ataxic with trajectories somewhere in between object location and the mirror. We called this group mirror ataxia and demonstrated that the lesions scattered around the anterior part of the intraparietal sulcus, whereas those of the mirror agnosia cases centered in the inferior posterior lobule. This aspect could not be compared with Ramachandran's patients, because their lesions were not shown.

When describing these 2 groups,[3] we had of course noticed the article of Ramachandran and colleagues that had appeared in the meantime. Accordingly, we adopted their term mirror agnosia that we had not used in our first contribution. Thus we acknowledged Ramachandran's terminology. But we regarded—and still do—the above-mentioned features in our patients as representing new syndromes distinctly different from that described by Ramachandran and colleagues by the reasons given above. If Altschuler now proposes to call mirror agnosia the Ramachandran sign, the authors should clarify in future work whether this refers to the

association of right parietal damage and left-sided visual hemineglect with mirror agnosia or to mirror agnosia per se.

Regarding our contribution,[3] we envision these data as disorders of the adjustment of separate body- and world-referenced representations—a concept that was elaborated experimentally in the primate parietal cortex.[4] According to the modular architecture of parietal cortex subserving cognitive processes and sensorimotor and coordinate transformations, a broad and variable range of visuospatial and visuo-motor disturbances can develop depending on the individual pathology. We need clear descriptions of these different facets. But in view of the range of possible combinations, delineations, and definitions, an attribution to names should be handled with care. What is pivotal is the usefulness of these facets for topological diagnosis and for the elucidation of structure-function relationships in the human brain.

Department of Neurology,
Heinrich-Heine-University Düsseldorf,
Düsseldorf, Germany

References

1. Ramachandran VS, Altschuler EL, Hillyer S. Mirror agnosia. Proc R Soc Lond B 1997;264:645–647
2. Binkofski F, Seitz RJ, Dohle C, et al. Dissociation of retinotopic space and proprioceptive body scheme: characterization of a parietal cortex lesion. Neurosci Abst. 1997;23:2374
3. Binkofski F, Buccino G, Dohle C, et al. Mirror agnosia and mirror ataxia constitute different parietal lobe disorders. Ann Neurol 1999;46:51–61
4. Snyder LH, Grieve KL, Brotchie P, Andersen RA. Separate body- and world-referenced representations of visual space in parietal cortex. Nature 1998;294:887–891

Corrections

The affiliation that accompanied Dr Dubowitz's editorial in the February issue (Ann Neurol 2000;47:143–144) was incorrect. His correct affiliation is as follows:

Victor Dubowitz, MD, PhD
Dubowitz Neuromuscular Centre
Department of Paediatrics and Neonatal Medicine
Imperial College of Science and Medicine
Hammersmith Campus
London, UK

In Table 1 of the article by Ohno et al in the February issue (Ann Neurol 2000;47:162–170), the nucleotide change for Patient 7 was incorrect. The correct nucleotide change is **1229G→A**.

The publisher apologizes for these errors.