

Cerebral Folate Deficiency and Leukoencephalopathy Caused by a Mitochondrial DNA Deletion

Merce Pineda, MD,¹ Aida Ormazabal, BS,¹ Esther López-Gallardo, BS,² Andres Nascimento, MD,¹ Abelardo Solano, PhD,² Maria D. Herrero, BS,² Maria A. Vilaseca, PhD,¹ Paz Briones, PhD,³ Lourdes Ibáñez, MD,¹ Julio Montoya, PhD,² and Rafael Artuch, MD¹

Objective: Our aim was to describe a child with an incomplete form of Kearns–Sayre syndrome who presented profound cerebrospinal fluid (CSF) folate deficiency and his response to folic acid supplementation. **Methods:** CSF 5-methyltetrahydrofolate was analyzed by HPLC with fluorescence detection and mitochondrial DNA deletions by southern blot hybridization. **Results:** Cranial magnetic resonance imaging showed a leukoencephalopathy. Profound CSF 5-methyltetrahydrofolate deficiency was observed with normal blood folate values and decreased CSF/serum folate ratio, suggesting a transport defect across the blood–brain barrier. Folic acid treatment was established, and after 1 year clinical response to folic acid supplementation was remarkable, with almost normal white matter image. **Interpretation:** The clinical response after folic acid therapy highlights the need for the study of cerebral folate deficiency in patients with mitochondrial disorders and white matter lesions.

Ann Neurol 2006;59:394–398

Mitochondrial DNA (mtDNA) deletion syndromes comprise three overlapping phenotypes: Pearson's syndrome, progressive external ophthalmoplegia, and Kearns–Sayre syndrome (KSS). KSS is a multisystemic disorder defined by onset before age 20 years, pigmentary retinopathy, progressive external ophthalmoplegia, and appearance of more clinical signs: cardiac conduction blockade, cerebrospinal fluid (CSF) protein concentration greater than 100mg/dl, and

From ¹Servicios de Neuropediatría, Bioquímica y Endocrinología, Hospital Sant Joan de Déu, Clínica; ²Departamento de Bioquímica y Biología Molecular y Celular, Universidad de Zaragoza; and ³Instituto de Bioquímica Clínica Corporació Sanitària y CSIC, Barcelona, Spain.

Received Jul 22, 2005, and in revised form Sep 14. Accepted for publication Oct 15, 2005.

Published online Dec 19, 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.20746

Address correspondence to Dr Pineda, Servicio Neuropediatría, Hospital Sant Joan de Déu, Passeig St. Joan de Déu no. 2, 08950 Esplugues, Barcelona, Spain. E-mail: pineda@hsjdbcn.org

cerebellar ataxia. Abnormalities in neuroradiological studies have been reported, affecting deep structures of the brain and subcortical white matter.¹ Mitochondrial DNA deletions, ranging in size from 2 to 10kb, are present.

There is no treatment for mitochondrial DNA deletion syndromes, and all therapeutic approaches have been unsuccessful. The association between cerebral folate deficiency (CFD) and KSS was first described in 1983, and clinical improvement after folic acid treatment was reported, but more than 20 years have elapsed since the last report of CFD in KSS.^{2,3}

Our aim was to describe a child with an incomplete form of KSS who presented profound CSF folate deficiency and his response to folic acid supplementation.

Case Report

This male patient was born of nonconsanguineous parents of Brazilian origin. At the age of 7 years and 8 months, he presented short stature and leukoencephalopathy was shown on cranial magnetic resonance imaging (MRI; Fig 1A, B). In the 2 months prior, the parents had noted an odd clumsy performance, poor coordination, strength gait deterioration, and low school level. Action tremor, slight dysmetria, slow speech, and brisk tendon reflexes were documented. Normal sensitivity for modalities was obtained. In the following months, progressive muscle weakness, fatigability, gait ataxia, flaccid tetraparesis, and areflexia appeared, with loss of deambulation at 8 years of age. A second brain MRI performed with spectroscopy showed diffuse leukodystrophy of the white matter of the subcortical regions in the upper frontal and parietal regions and, with spectroscopy, a peak of lactate with normal amount of *N*-acetylaspartate. Basal ganglia and cerebellum did not show abnormalities. Neurophysiological studies showed demyelinating neuropathy (motor velocity conduction: 37m/seg); visual evoked potentials evidenced delayed latency. Electroencephalogram and electroretinogram, fundus oculi, and cardiological examination were normal.

Laboratory Studies

Blood and CSF lactate, pyruvate, and amino acids were analyzed as previously reported.^{4,5} CSF 5-methyltetrahydrofolate (5-MTHF), 5-hydroxyindoleacetic, and homovanilic acids, and neopterin and biopterin concentrations were analyzed by high-performance liquid chromatography with electrochemical and fluorescence detection.^{6,7}

Histochemical studies of mitochondrial oxidative phosphorylation in muscle were performed with standard procedures. The oxidation rates of mitochondrial substrates and the activities of mitochondrial respiratory chain enzymes were determined by radiometric or spectrophotometric methods.^{8–10}

Genetic Studies

Total DNA was isolated from muscle and fibroblasts and was analyzed by Southern blot. To precisely map the position of the deletion, we performed complete mitochondrial

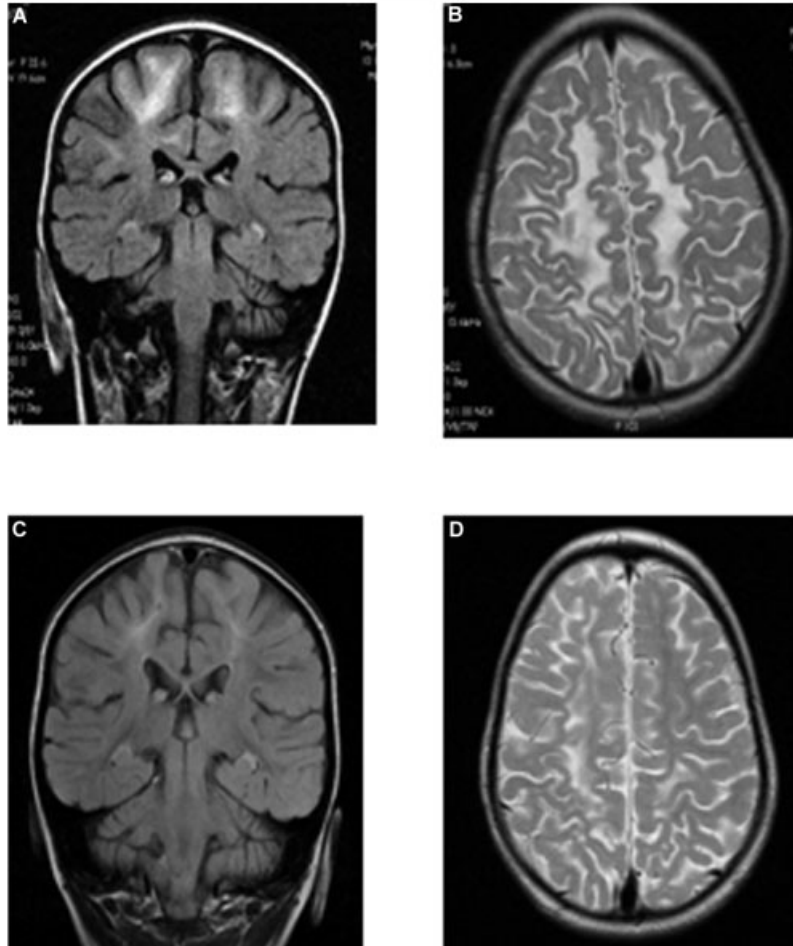


Fig 1. Brain MRI at 8 years of age before treatment with folic acid. FLAIR.T2. (A) Coronal image shows hyperintensity sparing the subcortical white matter with slightly periventricular location. (B) On axial T2 weighted image high intensity in the parietal white matter. After one year's treatment with folic acid (C) Coronal and (D): axial images show decreased signal on the same location.

genome amplification by long-range polymerase chain reaction. Restriction mapping allowed the identification of the region involved in the deletion, which then was amplified and sequenced. Deletion breakpoints were identified by comparing with the reference sequence for human mtDNA Cambridge Reference Sequence (CRS).

Samples from patients and controls were obtained in accordance with the 2000 revised Helsinki Declaration of 1975. Informed consent was obtained from the family. The Ethical Committee of the Sant Joan de Déu Hospital approved the study.

Results

Relevant biochemical results in blood and CSF are reported in the Table. In baseline conditions, increased lactate and alanine concentrations supported the diagnosis of a mitochondrial disorder. Serum folate concentration was within the reference range. Furthermore, no hyperhomocysteinemia or megaloblastic anemia were demonstrated.

In CSF, high protein and lactate concentrations were

observed. There was profound 5-MTHF deficiency, but dopamine and serotonin metabolites (5-hydroxyindoleacetic and homovanilic acids) and pterins were not decreased compared with our reference values (see Table). The ratio of CSF/serum folate was decreased (0.6: reference values, 1.5–3.0).

Muscle biopsy analysis showed ragged-red fibers (RRFs), cytochrome *c* oxidase (COX)–negative fibers, deficient pyruvate and malate oxidation, and partial deficiency of complex I and complex III activities of the mitochondrial respiratory chain.

Muscle and cultured fibroblasts mtDNA showed the presence of a single deletion of 4,123bp in 73 and 40% of the genome, respectively (Fig 2A, B). This deletion led to the loss of four polypeptide-coding genes (ND4, ND5, ND6, cytochrome *b*) and four tRNA genes (His, Ser, Leu[CUN], and Glu; see Fig 2C). Sequence analysis of the deletion breakpoints showed that the deletion was flanked by an 18/15 imperfect tandem repeat element (see Fig 2D).

Table. Biochemical Results in Blood and Cerebrospinal Fluid

	Baseline	First Control	Second Control	Ref. Values
Blood				
Lactate	2.50	3.3	1.27	0.55–1.80mmol/L
Pyruvate	0.10	0.12	0.10	0.03–0.1mmol/L
Alanine	470	666	503	185–410μmol/L
Carnitine	19	40	63	25.5–48μmol/L
Folate	14.2	52	66	9–41nmol/L
CSF				
Lactate	5.16	—	n.d.	1.1–2.2mmol/L
Alanine	42	—	n.d.	13–52μmol/L
5-HIAA	474	—	420	87–366nmol/L
HVA	844	—	872	202–596nmol/L
NP	55	—	37	10–46nmol/L
BP	32	—	20	8.2–68.1nmol/L
5-MTHF	8	—	48	45–94nmol/L

Biochemical results in blood and CSF in baseline conditions (before the start of therapy) and after folinic acid supplementation (first and second controls). CSF was taken only in the second control, 1 year after the start of the therapy.

CSF = cerebrospinal fluid; n.d. = not determined; 5-HIAA = 5-hydroxyindoleacetic; HVA = homovanilic acid; NP = neopterin; BP = biopterin; 5-MTHF = 5-methyltetrahydrofolate.

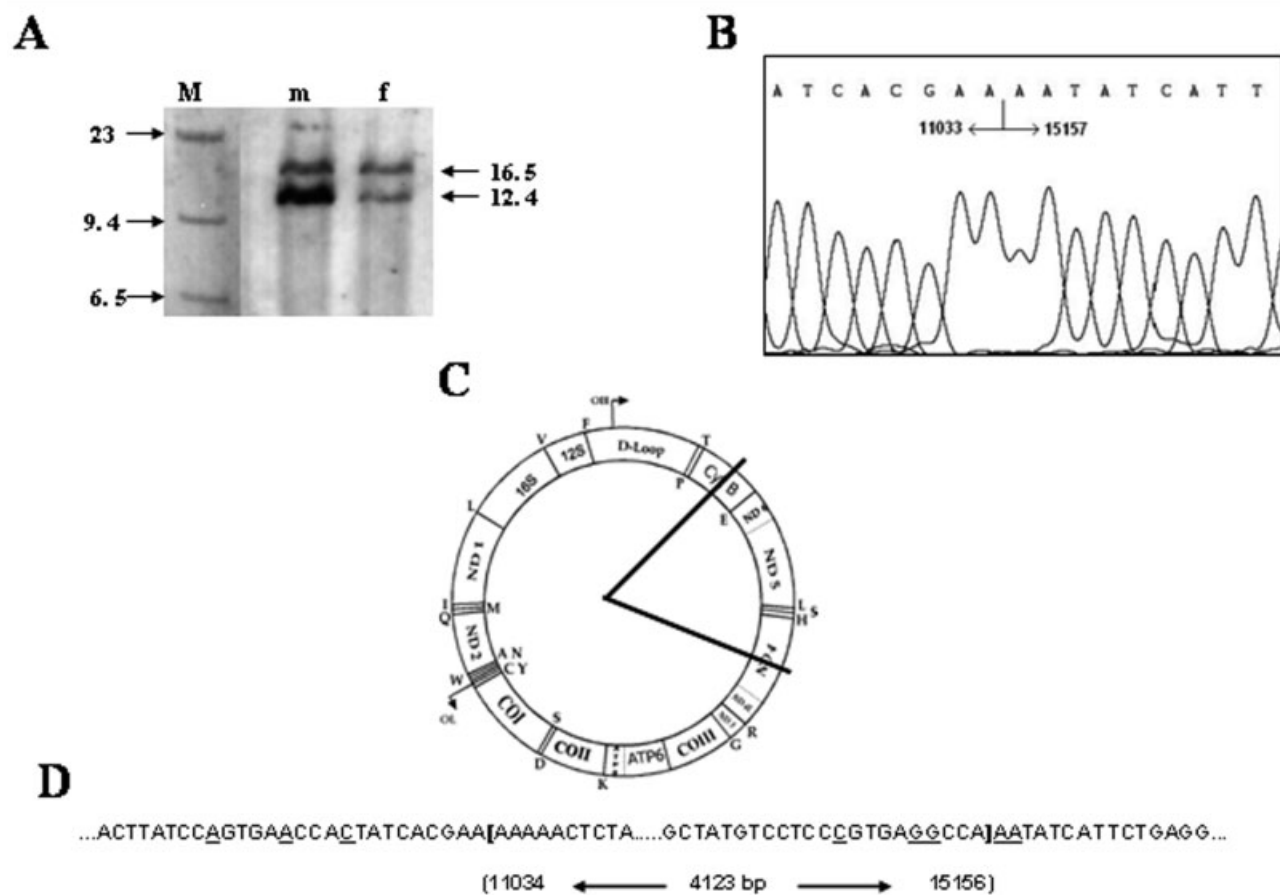


Fig 2. (A) Hybridization of the patient's muscle and fibroblast DNA with a human mtDNA probe (m: muscle; f: fibroblast; M: Molecular weight marker; numbers indicate kilobases). (B) Deletion breakpoints defined by automated sequencing. (C) Schematic representation of the deleted region throughout the entire human mtDNA molecule. (D) Analysis of the mtDNA sequence flanking the deletion breakpoints. Imperfect direct sequences flanking the breakpoints are shown in bold; the mismatched nucleotides are underlined.

Evolution

We started oral monotherapy with folic acid (1mg/kg/day) with doses increasing up to 2.5mg/kg/day in the following weeks. After 1 month of treatment, the patient recovered ambulation with independent ataxic gait and asymmetrical cerebellar syndrome on upper limbs. After 1 year of treatment with folic acid, a second determination in CSF showed normal 5-MTHF values, although the CSF/serum folate ratio remained below reference values (0.75; reference values, 1.5–3.0). At that moment, cranial MRI, T2, and fluid-attenuated inversion recovery sequences images (see Fig 1C, D) showed a great improvement in myelination compared with baseline conditions (see Fig 1A, B). No improvement was observed in cerebellar signs and areflexia.

Discussion

Mitochondrial DNA deletion syndromes might be difficult to recognize, especially in the first decade of life. Our patient showed cerebellar ataxia, impaired intellect, exercise intolerance, proximal muscle weakness, and leukoencephalopathy on brain MRI. Although our patient showed several symptoms related with KSS, he did not fulfill the main clinical criteria for this disorder, probably because he was still too young to develop the whole clinical picture. Taken together, the presence of cerebellar syndrome, demyelinating neuropathy, increased lactate and CSF protein values, RRF and COX-negative fibers in muscle biopsy, and large deletion in mtDNA suggest a diagnosis of KSS.

Brain MRI abnormalities are a frequent finding in mitochondrial cytopathies. Widespread white matter and cortical hyperintensity on T2-weighted images and in fluid-attenuated inversion recovery sequences can be observed. Supratentorial and infratentorial cortical atrophy and cerebellum atrophy observed in KSS did not appear in our patient, possibly because of his young age.¹¹ The highlighting image after 1 year of folic treatment led us to associate white matter alterations observed in our case with folic acid deficiency.

CFD syndrome is a disorder that may be defined “as any neurological syndrome associated with low CSF 5-MTHF values,” the active form of folate.¹² Some of the clinical features described in our case (cerebellar syndrome, white matter lesions, cognitive impairment, and spastic paraparesis) may also be observed in CFD. Cerebral folate deficiency occurs in the presence of normal blood folate concentration.¹² Therefore, the occurrence of CFD has been associated with a dysfunction in the high-affinity folate receptors allocated in the blood–brain barrier. Interestingly, CFD in KSS was demonstrated in three reports several years ago,^{2,3} and, according to the biochemical data reported, impairment in transport was possible. Our patient showed a normal blood folate status with a profound CSF folate deficiency, suggesting a trans-

port defect across the blood–brain barrier. Recently, we studied another patient with KSS, and a profound CSF 5-MTHF with normal blood folate status was also observed (data not shown). The presence of abundant Δ -mtDNAs in the choroids plexus of KSS patients has been reported,¹³ suggesting a key role in causing the reduced CSF folic acid values in this disorder.

5-MTHF is the precursor of the methyl-group donor *S*-adenosylmethionine, which is used in more than 100 chemical reactions. The methylation of arginine at position 107 within myelin basic protein is necessary to maintain stability of central nervous system myelin, and this might be a key factor in the central nervous system involvement in this case.¹⁴ Other metabolic pathways, such as biosynthesis of tetrahydrobiopterin, are also dependent on 5-MTHF,¹⁵ and a reduction in CSF concentrations of this cofactor has been associated with CFD.¹⁶ This reduction might be associated with low 5-hydroxyindoleacetic and homovanilic acids concentrations.¹⁶ According to our results, no decreased concentrations of these metabolites were observed, suggesting that the involvement of a potential neurotransmitter deficiency in our case would be less important than those affecting other folate functions, such as the methylation of the myelin basic protein.

Cerebral folate deficiency can induce abnormalities in cerebral white matter, and the clinical response to folic acid supplementation is remarkable.^{14,17} An important feature in our patient is an almost normal white matter image after folic monotherapy, and improvement on clinical grounds (the patient recovered deambulation and quality of life). Although the relationship between neurological manifestations and CSF folate deficiency in KSS is unknown, it is probable that folic acid treatment may correct these aspects. Nevertheless, a larger follow-up study seems necessary to establish the evolution of the disease after folic therapy. Moreover, it has been reported that mtDNA deletions may be reduced by folate supplementation in the liver of rats.¹⁸ The analysis of mtDNA deletions after folic acid therapy might be interesting to establish a link between folic acid therapy and clinical improvement of KSS patients.

In conclusion, these findings suggest that cerebral folate deficiency should be studied in patients with mitochondrial disorders and white matter lesions. The mechanisms involved in the cerebral folate deficiency in Kearns–Sayre syndrome are still unknown, although the clinical response after folic therapy strongly highlights the need for its study in mitochondrial disorders.

This study was supported by grants from the Diputación General de Aragón (Grupos Consolidados B33, E.L.-G., M.D. H., A.S., J. M.),

References

1. Zeviani M, Moraes CT, DiMauro S, et al. Deletions of mitochondrial DNA in Kearns-Sayre syndrome. *Neurology* 1988;38:1339–1346.
2. Allen J, DiMauro S, Coulter D, et al. Kearns-Sayre syndrome with reduced plasma and cerebrospinal fluid folate. *Ann Neurol* 1983;13:6:679–681.
3. Dougados M, Zittoun J, Laplane D, Castaigne P. Folate metabolism disorder in Kearns-Sayre syndrome. *Ann Neurol* 1983;13:679–682.
4. Artuch R, Vilaseca MA, Farré C, Ramón F. Determination of lactate, pyruvate, B-hydroxybutyrate and acetoacetate with a centrifugal analyser. *Eur J Clin Chem Clin Biochem* 1995;33:529–533.
5. Moyano D, Vilaseca MA, Artuch R, Lambuschini N. Plasma amino acids in anorexia nervosa. *Eur J Clin Nutr* 1998;52:684–689.
6. Belz S, Frickel C, Wolfrom C, et al. High-performance liquid chromatographic determination of methotrexate, 7-hydroxymethotrexate, 5-methyltetrahydrofolic acid and folic acid in serum and cerebrospinal fluid. *J Chromatogr B Biomed Appl* 1994;661:109–118.
7. Ormazabal A, García-Cazorla A, Fernández Y, et al. HPLC with electrochemical and fluorescence detection procedures for the diagnosis of inborn errors of biogenic amines and pterins. *J Neurosci Methods* 2005;142:153–158.
8. Rustin P, Chretien D, Bourgeron T, et al. Biochemical and molecular investigations in respiratory chain deficiencies. *Clin Chim Acta* 1994;228:35–51.
9. Fischer JC, Ruitenbeek W, Berden JA, et al. Differential investigation of the capacity of succinate oxidation in human skeletal muscle. *Clin Chim Acta* 1985;153:23–36.
10. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:265–275.
11. Barragan-Campos HM, Vallee JN, Lo D, Barrera-Ramirez CF, et al. Brain magnetic resonance imaging findings in patients with mitochondrial cytopathies. *Arch Neurol* 2005;62:737–742.
12. Ramaekers VT, Blau N. Cerebral folate deficiency. *Dev Med Child Neurol* 2004;46:843–851.
13. Tanji K, Schon EA, DiMauro S, Bonilla E. Kearns-Sayre syndrome: oncocyctic transformation of choroids plexus epithelium. *J Neurol Sci* 2000;178:29–36.
14. Surtees R, Leonard J, Austin S. Association of demyelination with deficiency of cerebrospinal-fluid S-adenosylmethionine in inborn errors of methyl-transfer pathway. *Lancet* 1991;338:1550–1554.
15. Ramaekers VT, Rothenberg SP, Sequeira JM, et al. Autoantibodies to folate receptors in the cerebral folate deficiency syndrome. *N Engl J Med* 2005;352:1985–1991.
16. Surtees R, Heales S, Bowron A. Association of cerebrospinal fluid deficiency of 5-methyltetrahydrofolate, but not S-adenosylmethionine, with reduced concentrations of the acid metabolites of 5-hydroxytryptamine and dopamine. *Clin Sci* 1994;86:697–702.
17. Hansen FJ, Blau N. Cerebral folate deficiency: life-changing supplementation with folic acid. *Mol Genet Metab* 2005;84:371–373.
18. Crott JW, Choi SW, Branda RF, Mason JB. Accumulation of mitochondrial DNA deletions is age, tissue and folate-dependent in rats. *Mut Res* 2005;570:63–70.

Spastic Paraplegia Type 2 Associated with Axonal Neuropathy and Apparent *PLP1* Position Effect

Jennifer A. Lee, BS,¹ Ricardo E. Madrid, MD,² Karen Sperle, MS,³ Carolyn M. Ritterson, BS,⁴ Grace M. Hobson, PhD,^{3,5} James Garbern, MD, PhD,⁶ James R. Lupski, MD, PhD,^{1,7,8} and Ken Inoue, MD, PhD^{1,9}

Objective: To report an association between spastic paraplegia type 2 with axonal peripheral neuropathy and apparent proteolipid protein gene (*PLP1*) silencing in a family. **Methods:** Pulsed-field gel electrophoresis, custom array comparative genomic hybridization, and semi-quantitative multiplex polymerase chain reaction analyses were used to examine the *PLP1* genomic region. **Results:** Electrodiagnostic studies and a sural nerve biopsy showed features of a dystrophic axonal neuropathy. Molecular studies identified a small duplication downstream of *PLP1*. **Interpretation:** We propose the duplication to result in *PLP1* gene silencing by virtue of a position effect. Our observations suggest that genomic rearrangements that do not include *PLP1* coding sequences should be considered as yet another potential mutational mechanism underlying *PLP1*-related dysmyelinating disorders.

Ann Neurol 2006;59:398–403

Pelizaeus–Merzbacher disease (PMD) and spastic paraplegia type 2 (SPG2) are clinically distinct allelic disorders, yet represent a wide spectrum of central ner-

From the ¹Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; ²New York State Institute for Basic Research in Developmental Disabilities, George A. Jervis Clinic, Staten Island, NY; ³Nemours Biomedical Research, Alfred I. duPont Hospital for Children, Nemours Children's Clinic, Wilmington, DE; ⁴Department of Biology, Boston University, Boston, MA; ⁵Department of Pediatrics, Jefferson Medical College, Philadelphia, PA; ⁶Department of Neurology and Center of Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI; ⁷Department of Pediatrics, Baylor College of Medicine and ⁸Texas Children's Hospital, Houston, TX; and ⁹Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan.

Received Mar 23, 2005, and in revised form Aug 24 and Sep 28. Accepted for publication Sep 29, 2005.

Published online Dec 22, 2005, in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.20732

Address correspondence to Dr Inoue, Department of Mental Retardation and Birth Defect Research National, Institute of Neurology National Center of Neurology and Psychiatry (NCNP), 4-1-1 Ogawahigashi, Kodaira, Tokyo 187-8502. E-mail: kinoue@ncnp.go.jp