infantile form of atypical NKH. The mutations are not detected in control subjects from the same geographic origin indicating that they are not prevalent polymorphisms [11,32]. When, these mutations were transfected into COS7 cells in vitro, a significant higher glycine decarboxylase activity was expressed compared with the classical NKH Finnish mutation. Mutant *GLDC* with the A389V and R739H mutations showed 6–8% of the wild-type glycine decarboxylase activity and that with the A802V mutation had 32% activity. Clinically, the A802V mutation is associated with a *neonatal* form of NKH and transient or absent symptoms with early therapeutic intervention. Two other missense mutations (R790W and L82W) on *GLDC* gene were found in two compound heterozygotes with *neonatal* form of atypical NKH [23]. Both mutations had a substantial higher glycine decarboxylase activity when expressed in COS7 cells (14 and 11% of the wild-type, respectively). Despite the limited data it seems that there is a genotype–phenotype correlation with certain mutations associated with a more favorable outcome. These mutations, even in a compound heterozygous state, permit some residual enzyme activity that may be responsible for the milder phenotype. Finally a pathogenic exonic *GLDC* splice mutation (silent transversion c.2607C>A), that does not change the amino acid sequence but does cause reduced *GLDC* mRNA levels on lymphoblast, was identified in homozygous state in a large kindred with atypical NKH [33].

Diagnosis and treatment

Identification of patients with atypical NKH will require increased clinical suspicion, especially in populations with non-specific mild mental retardation, in whom a laboratory work up is usually omitted. The presence of behavioral problems especially outbursts of rage and medically intractable ADHD, choreoathetosis, and ataxic episodes should raise suspicion for atypical NKH. Simultaneous plasma and CSF glycine measurements are required in patients with isolated elevations in urine or plasma glycine levels. The CSF/plasma glycine ratio (normal $\leqslant 0.02$) in atypical cases is usually lower than the diagnostic cut-point for classical NKH (0.08) but results require careful interpretation because other conditions and drugs can cause hyperglycinemia and hyperglycinorachia, and sampling errors may occur [34]. Difficulties also may arise in separating out the rare cases of transient neonatal NKH, so enzymatic or genetic confirmation is of paramount importance. Measurement of GCS activity can be done in liver tissue or transformed lymphoblasts [35]; the measurement of the liver GCS activity is more sensitive, but because it is more invasive, it should be reserved for the selected, indeterminate cases. Identification of the causative mutation helps for diagnostic confirmation, accurate genetic counseling, and prenatal diagnosis. Unfortunately there is not an immediately available test to predict with reliability the outcome. The expression analysis of the mutation can identify favorable mutations with residual enzyme activity that may help in future decision-making.

Ancillary testing such as EEG and brain imaging studies add little to the diagnosis of atypical NKH, since they show no specific abnormalities. Proton MR spectroscopy (^{1}HMRS) allows non-invasive measurement of glycine concentrations and has been described as a useful tool for diagnosis and monitoring the therapeutic interventions in classical NKH [36]. However, in three atypical cases (cases 11, 12, 20a) ^{1}HMRS failed to show increased glycine, which presumably reflects lower parenchymal brain glycine concentrations, below ^{1}HMRS detection level [11,32].

The traditional treatment of classical NKH involves agents that decrease glycine concentration, as well as agents that antagonize the overstimulation of NMDA receptor. In atypical NKH cases treatment such as low protein diet, sodium benzoate, dextromethorphan, strychnine, ketamine, tryptophan, and imipramine have been tried alone or in combination. Clinical improvement has been described but it is not consistent. Several investigators also used various single carbon donors (methionine, folate, and leucovorin) in an attempt to correct the presumed deficiency of single carbon units in NKH [37]. Overall, it is difficult to infer the value of treatment since all data are from small case series and the age of initiation of treatment varies. For specific "favorable" mutations the timing of therapeutic intervention is crucial, and NMDA receptors antagonists and glycine reducing agents may have a beneficial impact on the outcome.

Conclusions and future aspects

The identification of mutations on *GLDC* gene in patients with *neonatal* and *infantile* form of NKH demonstrates that this form represents a continuum with the classical NKH and that the milder atypical phenotype is presumably the result of the residual GCS function. The *late onset* NKH may represent a different entity and more studies are needed to identify the cause of the hyperglycinemia. The true incidence of atypical NKH is unknown; it is likely underdiagnosed. Since all patients have increased plasma and urine glycine, testing plasma, and urine amino acids in patients with mild mental retardation, particularly those with behavioral problems and abnormal movements or seizures, may be of diagnostic use. The real challenge is the early and reliable prediction of those patients who will have a mild phenotype and of those who an early therapy will

produce a favorable outcome before irreversible damage occurs. Identification of pathogenic mutations and information of their expression analysis, in a number of atypical NKH patients, may give us a more accurate genotype–phenotype correlation and help with the decision making in clinical setting.

Acknowledgment

We thank Dr. Mark Schapiro who reviewed and commented on the manuscript.

References

[1] M. Takayanagi, S. Kure, Y. Sakata, Y. Kurihara, Y. Ohya, M. Kajita, K. Tada, Y. Matsubara, K. Narisawa, Human glycine decarboxylase gene (GLDC) and its highly conserved processed pseudogene (psiGLDC): their structure and expression, and the identification of a large deletion in a family with nonketotic hyperglycinemia, Hum.Genet. 106 (2000) 298–305.

[2] K. Nanao, K. Okamura-Ikeda, Y. Motokawa, D.M. Danks, E.R. Baumgartner, G. Takada, K. Hayasaka, Identification of the mutations in the T-protein gene causing typical and atypical nonketotic hyperglycinemia, Hum. Genet. 93 (1994) 655–658.

[3] S. Kure, K. Kojima, T. Kudo, K. Kanno, Y. Aoki, Y. Suzuki, T. Shinka, Y. Sakata, K. Narisawa, Y. Matsubara, Chromosomal localization, structure, single-nucleotide polymorphisms, and expression of the human H-protein gene of the glycine cleavage system (GCSH), a candidate gene for nonketotic hyperglycinemia, J. Hum. Genet. 46 (2001) 378–384.

[4] S.W. Scherer, G. Otulakowski, B.H. Robinson, L.C. Tsui, Localization of the human dihydrolipoamide dehydrogenase gene (DLD) to 7q31-q32, Cytogenet. Cell Genet. 56 (1991) 176–177.

[5] K. Hiraga, H. Kochi, K. Hayasaka, G. Kikuchi, W.L. Nyhan, Defective glycine cleavage system in nonketotic hyperglycinemia. Occurrence of a less active glycine decarboxylase and an abnormal aminomethyl carrier protein, J. Clin. Invest. 68 (1981) 525–534.

[6] S. Kure, M. Takayanagi, K. Narisawa, K. Tada, J. Leisti, Identification of a common mutation in Finnish patients with nonketotic hyperglycinemia, J. Clin. Invest. 90 (1992) 160–164.

[7] J.R. Toone, D.A. Applegarth, M.B. Coulter-Mackie, E.R. James, Biochemical and molecular investigations of patients with nonketotic hyperglycinemia, Mol. Genet. Metab. 70 (2000) 116–121.

[8] D.A. Applegarth, J.R. Toone, Nonketotic hyperglycinemia (glycine encephalopathy): laboratory diagnosis, Mol. Genet. Metab. 74 (2001) 139–146.

[9] J.R. Toone, D.A. Applegarth, S. Kure, M. Coulter-Mackie, P. Sazegar, K. Kojima, A. Ichinohe, Novel mutations in the P-protein gene in patients with glycine encephalopathy (non-ketotic hyperglycinemia), Mol. Genet. Metab. 76 (2002) 243–249.

[10] T. Gerritsen, E. Kaveggia, H.A. Waisman, A new type of idiopathic hyperglycinemia with hypo-oxaluria, Pediatrics 36 (1965) 882.

[11] S.H. Korman, A. Boneh, A. Ichinohe, et al., Persistent NKH with transient or absent symptoms and a homozygous GLDC mutation, Ann. Neurol. 56 (2004) 139–143.

[12] A. Hamosh, J.W. McDonald, D. Valle, C.A. Francomano, E. Niedermeyer, M.V. Johnston, Dextromethorphan and high-dose benzoate therapy for nonketotic hyperglycinemia in an infant, J. Pediatr. 121 (1992) 131–135.

[13] D.E.C. Cole, D.C. Meek, Juvenile non-ketotic hyperglycinemia in three siblings, J. Inherit. Metab. Dis. 2 (1985) 123–124.

[14] E. Haan, D.M. Kirby, K. Tada, K. Hayasaka, D.M. Danks, Difficulties in assessing the effect of strychnine on the outcome of non-ketotic hyperglycinemia. Observations on sisters with a mild T-protein defect, Eur. J. Pediatr. 145 (1986) 267–270.

[15] D.A. Trauner, T. Page, C. Greco, L. Sweetman, S. Kulovich, W.L. Nyhan, Progressive neurodegenerative disorder in a patient with nonketotic hyperglycinemia, J. Pediatr. 98 (1981) 272–275.

[16] T. Hasegawa, Y. Shiga, A. Matsumoto, A. Takeda, Y. Itoyama, Late-onset nonketotic hyperglycinemia: a case report, No To Shinkei 54 (2002) 1068–1072.

[17] W.J. Bank, G. Morrow, Familial neuromuscular disease with nonketotic hyperglycinemia, Trans. Am. Neurol. Assoc. 96 (1971) 21–23.

[18] J.E. Hoover-Fong, S. Shah, J.L. Van Hove, D. Applegarth, J. Toone, A. Hamosh, Natural history of nonketotic hyperglycinemia in 65 patients, Neurology 63 (2004) 1847–1853.

[19] G. Holmgren, H. Kison Blomquist, Nonketotic hypergycinemia in two sibs with mild psycho-neurological development, Neuropadiatrie 8 (1977) 67–72.

[20] A.H. Jackson, D.A. Applegarth, J.R. Toone, S. Kure, H.L. Levy, Atypical nonketotic hyperglycinemia with normal cerebrospinal fluid to plasma glycine ratio, J. Child Neurol. 14 (1999) 464–467.

[21] D. Aliefendioglu, A. Tana Aslan, T. Coskun, A. Dursun, F.N. Cakmak, M. Kesimer, Transient nonketotic hyperglycinemia: two case reports and literature review, Pediatr. Neurol. 28 (2003) 151–155.

[22] K. Hayasaka, K. Tada, G. Kikuchi, S. Winter, W.L. Nyhan, Nonketotic hyperglycinemia: two patients with primary defects of P-protein and T-protein, respectively, in the glycine cleavage system, Pediatr. Res. 17 (1983) 967–970.

[23] S. Kure, A. Ichinohe, K. Kojima, K. Sato, Z. Kizaki, F. Inoue, C. Yamanaka, Y. Matsubara, Mild variant of nonketotic hyperglycinemia with typical neonatal presentations: mutational and in vitro expression analyses in two patients, J. Pediatr. 144 (2004) 827–829.

[24] H.S. Singer, D. Valle, K. Hayasaka, K. Tada, Nonketotic hyperglycinemia: studies in an atypical variant, Neurology 39 (1989) 286–288.

[25] M.A. Zorbin, J.K. Wamsley, M.J. Kiehor, Glycine receptor: light microscopic localization with ^{3}H-strychnine, J. Neurosci. 1 (1981) 532.

[26] A.A. Larson, A.J. Beitz, Glycine potentiates strychnine-induced convulsions: role of NMDA receptor, J. Neurosci. 8 (1988) 3822.

[27] D.M. Frazier, G.K. Summer, H.R. Chamberlin, Hyperglycinuria and hyperglycinemia in two siblings with mild developmental delays, Am. J. Dis. Child 132 (1978) 777–781.

[28] J.E. Wraith, Non-ketotic hyperglycinemia: prolonged survival in a patient with a mild variant, J. Inherit. Metab. Dis. 19 (1996) 695–696.

[29] R.G.M. Morris, Synaptic plasticity and learning in rats and blockade of long term potentiation in vivo by NMDA receptor antagonist AP5, J. Neurosci. 9 (1989) 3040–3057.

[30] A.P. Carobrez, K.V. Teixeira, F.G. Graeft, Modulation of defensive behavior by periaqueductal gray NMDA/glycine-B receptor, Neurosci. Behav. Rev. 25 (2001) 697–709.

[31] A. Ichinohe, S. Kure, S. Mikawa, T. Ueki, K. Kojima, K. Fujiwara, K. Iinuma, Y. Matsubara, K. Sato, Glycine cleavage system in neurogenic regions, Eur. J. Neurosci. 19 (2004) 2365–2370.

[32] A. Dinopoulos, S. Kure, G. Chuck, K. Sato, D.L. Gilbert, Y. Matsubara, T. Degrauw, Glycine decarboxylase mutations: a distinctive phenotype of nonketotic hyperglycinemia in adults, Neurology 64 (2005) 1255–1257.

[33] H. Flusser, S.H. Korman, K. Sato, Y. Matsubara, A. Galil, S. Kure, Mild glycine encephalopathy (NKH) in a large kindred due to a silent exonic GLDC splice mutation, Neurology 64 (2005) 1426–1430.

[34] S.H. Korman, A. Gutman, Pitfalls in the diagnosis of glycine encephalopathy (non-ketotic hyperglycinemia), Dev. Med. Child Neurol. 44 (2002) 712–720.
[35] S. Kure, K. Narisawa, K. Tada, Enzymatic diagnosis of nonketotic hyperglycinemia with lymphoblasts, J. Pediatr. 120 (1992) 95–98.
[36] A. Viola, B. Chabrol, F. Nicoli, S. Confort-Gouny, P. Viout, P.J. Cozzone, Magnetic resonance spectroscopy study of glycine pathways in nonketotic hyperglycinemia, Pediatr. Res. 52 (2002) 292–300.
[37] A. Hamosh, M.V. Johnston, C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle, Nonketotic Hyperglycinemia, The Metabolic and Molecular Bases of Inherited Disease, McGraw-Hill, New York, 2001.
[38] R.D. Steiner, D.A. Sweetser, J.R. Rohrbaugh, S.B. Dowton, J.R. Toone, D.A. Applegarth, Nonketotic hyperglycinemia: atypical clinical and biochemical manifestations, J. Pediatr. 128 (1996) 243–246.
[39] J.M. Neuberger, S. Schweitzer, M.O. Rolland, R. Burghard, Effect of sodium benzoate in the treatment of atypical nonketotic hyperglycinaemia, J. Inherit. Metab. Dis. 23 (2000) 22–26.
[40] J. Christodoulou, S. Kure, K. Hayasaka, J.T. Clarke, Atypical nonketotic hyperglycinemia confirmed by assay of the glycine cleavage system in lymphoblasts, J. Pediatr. 123 (1993) 100–102.
[41] K. Hayasaka, K. Tada, N. Fueki, Y. Nakamura, W.L. Nyhan, K. Schmidt, S. Packman, M.R. Seashore, E. Haan, D.M. Danks, et al., Nonketotic hyperglycinemia: analyses of glycine cleavage system in typical and atypical cases, J. Pediatr. 110 (1987) 873–877.
[42] C. Borrone, C. Bachmann, M. DiRocco, K. Tada, K. Hayasaka, R. Gatti, Atypical non-ketotic hyperglycinemia, J. Inherit. Metab. Dis. 9 (1986) 402–403.
[43] Y. Tanaka, M. Miyazaki, M. Tsuda, K. Murai, S. Kuzuhara, Blindness due to non-ketotic hyperglycinemia: report of a 38-year-old, the oldest case to date, Intern. Med. 32 (1993) 641–642.
[44] D.A. Hall, S.P. Ringel, Adult nonketotic hyperglycinemia (NKH) crisis presenting as severe chorea and encephalopathy, Mov. Disord. 19 (2004) 485–486.
[45] E.J. Wiltshire, N.K. Poplawski, J.R. Harrison, J.M. Fletcher, Treatment of late-onset nonketotic hyperglycinaemia: effectiveness of imipramine and benzoate, J. Inherit. Metab. Dis. 23 (2000) 15–21.
[46] G.S. Steiman, M. Yudkoff, P.H. Berman, B. Blazer-Yost, S. Segal, Late-onset nonketotic hyperglycinemia and spinocerebellar degeneration, J. Pediatr. 94 (1979) 907–911.
[47] D.B. Flannery, J. Pellock, D. Bousounis, P. Hunt, C. Nance, B. Wolf, Nonketotic hyperglycinemia in two retarded adults: a mild form of infantile nonketotic hyperglycinemia, Neurology 33 (1983) 1064–1066.

based on a thorough search of the medical literature and a poison control center consultation. Tamoxifen infrequently causes reversible neurotoxicity, but only at much higher doses ($\geq$160mg/m^2 daily).[5]

Recent investigations into the use of artemisinin compounds in cancer treatment[6] have not been substantiated in clinical trials. Because of pervasive reports of animal brainstem toxicity, and the gradual emergence of similar patient cases, it becomes imperative to ascertain the safety of prolonged courses of artemisinin for cancer prophylaxis.

[1]School of Medicine and [2]Department of Neurology, University of California–San Francisco, San Francisco, CA

References

1. Kissinger E, Hien TT, Hung NT, et al. Clinical and neurophysiological study of the effects of multiple doses of artemisinin on brain-stem function in Vietnamese patients. Am J Trop Med Hyg 2000;63:48–55.
2. Miller LG, Panosian CB. Ataxia and slurred speech after artesunate treatment for falciparum malaria. N Engl J Med 1997;336: 328–1329.
3. Brewer TG, Grate SJ, Peggins JO, et al. Fatal neurotoxicity of arteether and artemether. Am J Trop Med Hyg 1994;51: 251–259.
4. Schmuck G, Roehrdanz E, Haynes RK, Kahl R. Neurotoxic mode of action of Artemisinin. Antimicrob Agents Chemother 2002;46:821–827.
5. Bergan RC, Reed E, Myers CE, et al. A phase II study of high-dose tamoxifen in patients with hormone-refractory prostate cancer. Clin Cancer Res 1999;5:2366–2373.
6. Singh NP, Lai H. Selective toxicity of dihydroartemisinin and holotransferrin toward human breast cancer cells. Life Sci. 2001; 70:49–56.

DOI: 10.1002/ana.20620

Adult Alexander's Disease without Leukoencephalopathy

Fabrizio Salvi, MD, PhD,[1] Yoko Aoki, MD, PhD,[2] Riccardo Della Nave, MD,[3] Alessandra Vella, MD,[4] Francesca Pastorelli, MD,[1] Cesa Scaglione, MD,[1] Yoichi Matsubara, MD,[2] and Mario Mascalchi, MD, PhD[3]

Diagnosis of Alexander's disease is based on demonstration of Rosenthal fibers in astrocytes but can be supported by detection of mutations in the gene encoding the glial fibrillary acidic protein (GFAP).[1,2] Three clinical subtypes of Alexander's disease are identified, namely, infantile, juvenile, and adult, which usually occur sporadically, arising through de novo mutations.[2]

A 71-year-old man was referred for a complex neurological syndrome that began at age 52 years with muscle pain and unsteady gait. At age 61 years, he developed progressive lower limb weakness, dysarthria, and dysphagia. Neurological examination showed palatal myoclonus, gaze-evoked nystagmus, cogwheel phenomenon on smooth pursuit, diffuse bradykinesia, and dysmethria and bilateral Babinski sign. Magnetic resonance imaging (MRI; Fig) showed thinning of the medulla and cervical spinal cord and a cross-like hyperintensity in the medulla in T2-weighted images. Few small foci of

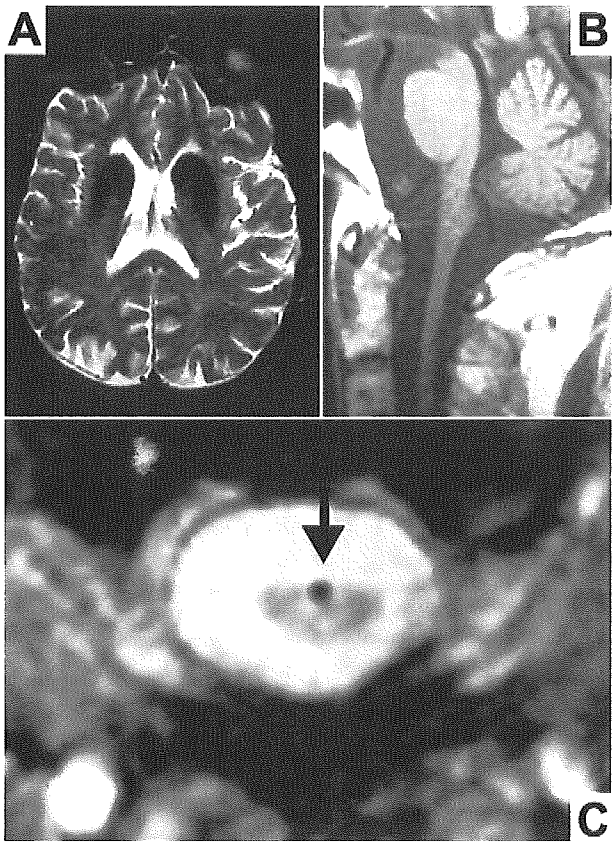

Fig. Axial T2-weighted (TR, 2,400; TE, 100) (A) spin-echo image shows symmetric hypointensity of the caudate and putamen and along the lateral ventricular walls without signal abnormality of the periventricular white matter. Sagittal T1-weighted (TR, 500; TE, 14) (B) spin-echo image shows marked thinning of the medulla and of the upper cervical spinal cord. Axial T2-weighted gradient-echo image (TR, 500; flip angle, 20 degrees; TE, 25) of the spine at C2 (C) demonstrates dot-like hypointensity* (arrow) *in the anterior sulcus of a thinned spinal cord.*

age-related signal changes were present in the cerebral white matter. A symmetric T2 hypointensity of the caudate and putamen was present, as well as a line of T2 hypointensity along the walls of the lateral ventricles and in the anterior sulcus of the cervical spinal cord. Molecular investigation[1] showed a C to T substitution at nucleotide 208 in exon 1 of the *GFAP* gene in the heterozygous form resulting in change of arginine to tryptophan at position 70 (R70W).

The prominent MRI finding in our patient was atrophy of the medulla and cervical spinal cord. The linear T2 hypointensity along the lateral ventricular walls and in the anterior sulcus of the spinal cord might be related to accumulation of Rosenthal fibers in these cerebrospinal fluid recesses.[3] Interestingly, small heat shock proteins and ubiquitin, which with GFAP are components of Rosenthal fibers, are increased in the cerebrospinal fluid of patients with Alexander's disease.[3] We did not observe any leukoencephalopathy consistent with the view that Alexander's disease is not an obligate leukodystrophy; this is in line with adult Alexander's disease cases

previously reported.[4] However, the MRI features of adult Alexander's disease are not homogeneous. In fact, adults with GFAP mutation exhibiting leukoencephalopathy were reported.[5] We submit that the MRI features of Alexander's disease can range from a pure leukoencephalopathy (predominant in the infantile variant[3]) to a medullary-spinal cord atrophy without leukoencephalopathy. The mechanisms underlying this spectrum deserve further studies. Search of the GFAP mutation is recommended in adults with progressive bulbar palsy and atrophy of the medulla and the spinal cord.

[1]Division of Neurology Bellaria Hospital, University of Bologna, Italy; [2]Department of Medical Genetics, Tohoku University School of Medicine, Sendai, Japan; [3]Radiodiagnostic Section, Department of Clinical Physiopathology, University of Florence, Florence; and [4]Division of Nuclear Medicine, University of Siena, Siena, Italy

References

1. Brenner M, Johnson AB, Boespflug-Tanguy O, et al. Mutations in GFAP encoding glial fibrillary acidic protein are associated with Alexander disease. Nat Genet 2001;27:117–120.
2. Moser HW. Alexander disease: combined gene analysis and MRI clarify pathogenesis and extend phenotype. Ann Neurol 2005;57: 307–308.
3. Van der Knaap MS, Naidu S, Breiter SN, et al. Alexander disease: diagnosis with MR imaging. AJNR Am J Neuroradiol 2001;22:541–552.
4. Namekawa M, Takiyama Y, Aoki Y, et al. Identification of GFAP gene mutation in hereditary adult-onset Alexander's disease. Ann Neurol 2002;52:779–785.
5. Li R, Johnson AB, Salomons G, et al. Glial fibrillary acidic protein mutations in infantile, juvenile, and adult forms of Alexander disease. Ann Neurol 2005;57:310–326.

Is Intracranial Monitoring Dispensable for Neocortical Epilepsy with Normal Magnetic Resonance Imaging?

Richard Wennberg, MD, FRCPC

I was interested to read in the article of Lee and colleagues[1] that no prognostic value could be attributed to the localization or morphology of ictal onsets recorded with subdural electrodes in their patients with neocortical epilepsy. This finding comes as no surprise to me. Notwithstanding, the authors repeat the current assumption that "intracranial monitoring is indispensable for neocortical epilepsy with normal MRI" and they do not follow through on their results by drawing this assumption of indispensability into question.

There is a modern paradox in the logical processes underlying the planning of surgical management in patients with neocortical epilepsy. As is typically the case, surgical resection in the patients described by Lee and colleagues[1] was guided by the margins of the "intracranial ictal onset zone," the same zone that the authors subsequently found to have no relation to surgical outcome. In fact, from the techniques reported, it was only the findings of noninvasive presurgical investigations (mainly interictal electroencephalography and positron emission tomography) that were independently related to surgical outcome.[1] All this is puzzling when one considers that the same noninvasive evaluations are used to determine the placements of the subdural electrodes for intracranial monitoring.[1]

From the results of studies of acute and chronic intracranial electrocorticography,[2,3] I have suggested previously that surgery for neocortical epilepsy could be contemplated without chronic invasive recording.[4] In that intracranial monitoring is expensive, time-consuming, and not without risk, our inability to clearly demonstrate a prognostic value in neocortical epilepsy should, at the very least, raise the question of dispensability in this particular setting.

Division of Neurology, University of Toronto, Toronto, Ontario, Canada

References

1. Lee SK, Lee SY, Kim K-K, et al. Surgical outcome and prognostic factors of cryptogenic neocortical epilepsy. Ann Neurol 2005; 58:525–532.
2. Wennberg R, Quesney F, Olivier A, Rasmussen T. Electrocorticography and outcome in frontal lobe epilepsy. Electroenceph Clin Neurophysiol 1998;106:357–368.
3. Bautista RED, Cobbs MA, Spencer DD, Spencer SS. Prediction of surgical outcome by interictal epileptiform abnormalities during intracranial EEG monitoring in patients with extrahippocampal seizures. Epilepsia 1999;40:880–890.
4. Wennberg R. Poor surgical outcome in patients with neocortical epilepsy is correlated with interictal epileptiform abnormalities outside the area of surgical resection. Epilepsia 2000;41: 355–357.

DOI: 10.1002/ana.20641

Reply

Necessity of Invasive Monitoring in Neocortical Epilepsy with Normal Magnetic Resonance Imaging

Sang Kun Lee, MD

Dr Wennberg suggested that surgery for neocortical epilepsy with normal magnetic resonance imaging (MRI) could be contemplated without chronic invasive recording. We think that this is an interesting and important point. Our results did not find any relationship between a specific intracranial ictal onset pattern, such as focal or regional onset, and surgical outcome. However, this does *not* mean that the resection of this zone did not relate to surgical outcome or that intracranial monitoring is dispensable for these patients. It only means that a specific onset pattern (the number of electrodes with intracranial ictal onset rhythm or the frequency of intracranial ictal onset rhythm) did not relate to surgical outcome. We resected the area with intracranial ictal onset irrespective of onset pattern, and many patients have benefited from this. Only by sparing some intracranial ictal onset area and showing a good surgical outcome could we prove that intracranial monitoring is indeed dispensable. However, we could not claim this because our resection margin always included the intracranial ictal onset zone.

The necessity of using intracranial electrodes depends on various situations and treatment policy. There should be pros and cons. However, we consider that intracranial monitoring

MUTATION UPDATE

Mutations in the Holocarboxylase Synthetase Gene *HLCS*

Yoichi Suzuki,* Xue Yang, Yoko Aoki, Shigeo Kure, and Yoichi Matsubara

Department of Medical Genetics, Tohoku University School of Medicine, Sendai, Japan

Communicated by Mark H. Paalman

Holocarboxylase synthetase (HLCS) deficiency is an autosomal recessive disorder. HLCS is an enzyme that catalyzes biotin incorporation into carboxylases and histones. Since the first report of the cDNA sequence, 30 mutations in the *HLCS* gene have been reported. Mutations occur throughout the entire coding region except exons 6 and 10. The types of mutations are one single amino acid deletion, five single nucleotide insertions/deletions, 22 missense mutations, and two nonsense mutations. The only intronic mutation identified thus far is c.1519+5G>A (also designated IVS10+5G>A), which causes a splice error. Several lines of evidence suggest that c.1519+5G>A is a founder mutation in Scandinavian patients. Prevalence of this mutation is about 10 times higher in the Faroe Islands than in the rest of the world. The mutations p.L237P and c.780delG are predominant only in Japanese patients. These are probably founder mutations in this population. Mutations p.R508W and p.V550M are identified in several ethic groups and accompanied with various haplotypes, suggesting that these are recurrent mutations. There is a good relationship between clinical biotin responsiveness and the residual activity of HLCS. A combination of a null mutation and a point mutation that shows less than a few percent of the normal activity results in neonatal onset. Patients who have mutant HLCS with higher residual activity develop symptom after the neonatal period and show a good clinical response to biotin therapy. Hum Mutat 26(4), 285–290, 2005. © 2005 Wiley-Liss, Inc.

KEY WORDS: holocarboxylase synthetase; HLCS; multiple carboxylase deficiency; biotin

INTRODUCTION

Holocarboxylase synthetase (HLCS; MIM# 609018; EC 6.3.1.10) is an enzyme that catalyzes biotin incorporation into carboxylases [Achuta Murthy and Mistry, 1972; Wolf, 2001]. In humans, four carboxylases are known to be biotinylated by HLCS. They are pyruvate carboxylase, propionyl-CoA carboxylase, and methylcrotonyl-CoA carboxylases, which are mitochondrial matrix enzymes, and acetyl-CoA carboxylase, located in both the cytosol and the mitochondrial membrane. There are two acetyl-CoA carboxylases genes. The ACACA gene (MIM# 200350) gene encodes only a cytosolic enzyme whereas the ACACB gene (MIM# 601557) gene produces both the cytosolic and the mitochondrial enzymes [Pacheco-Alvarez et al., 2002]. Biotinylation of these carboxylases is essential for their enzymatic activities. Recently, HLCS has been thought to biotinylate histones, although the physiological significance has not been elucidated [Narang et al., 2004; Peters et al., 2002; Stanley et al., 2001].

Holocarboxylase synthetase deficiency (MIM# 253270) is an autosomal recessive disorder. It is also called (biotin-responsive) multiple carboxylase deficiency (MCD), because deficient HLCS activity results in reduced activity of multiple carboxylases. The exact incidence of this disease is not known. Our laboratory has diagnosed one or two new cases per year in Japan, where approximately 1.2 million babies are born every year. We suspect the incidence of HLCS deficiency is less than 1 in 100,000 live births per year in Japan. Most patients with HLCS deficiency manifest symptoms in the newborn to early infantile period [Narisawa et al., 1982; Wolf, 2001]. Symptoms of HLCS deficiency include metabolic acidosis, a characteristic organic aciduria, lethargy, hypotonia, convulsions, and dermatitis. Many symptoms of HLCS are also seen in biotinidase deficiency (MIM# 253260), another type of MCD. Some patients become symptomatic in the later infantile period, at the age of several months to years [Chikaoka et al., 1992; Gibson et al., 1996; Suormala et al., 1997, 1998]. All patients with HLCS deficiency reported thus far have responded to biotin administration. In some patients, however, the response was only partial as manifested by continued excretion of abnormal metabolites in the urine. Developmental abnormalities have also been reported in some cases in spite of high-dose biotin therapy [Baumgartner and Suormala, 1997; Santer et al., 2003; Suzuki et al., 1996; Wolf et al., 1981].

Received 8 October 2004; accepted revised manuscript 24 March 2005.

*Correspondence to: Yoichi Suzuki, Department of Medical Genetics, Tohoku University School of Medicine, 1-1 Seiryomachi, Aobaku, Sendai 980-8574, Japan.

Current address: Department of Public Health, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku Chiba, 260-8670 Japan. E-mail: ysuzuki@faculty.chiba-u.jp

Grant sponsor: Grant-in-Aid for Scientific Research, Ministry of Education, Culture, Sports, Science and Technology of Japan; Grant sponsor: Ministry of Health, Labor, and Welfare of Japan.

DOI 10.1002/humu.20204

Published online in Wiley InterScience (www.interscience.wiley.com).

BIOLOGICAL RELEVANCE

Isolation of human cDNA for HLCS from the liver enabled the investigation of HLCS at the molecular level [Leon-Del-Rio et al., 1995; Suzuki et al., 1994]. The cDNA encoded 726 amino acids with in-frame stop codons lying at 5′ upstream of the first methionine. The deduced protein sequence contains a homologous region (amino acids numbers 448–701) to BirA, the biotin apo-carboxyl carrier protein ligase of *Escherichia coli*. This homologous portion of human HLCS is thought to be the putative biotin-binding region [Suzuki et al., 1994]. The importance of the domain outside the biotin-binding region of HLCS for enzymatic activity was examined in an expression study with HLCS deletion mutants. The N-terminal amino acid region up to Ile117 had much less effect on the enzymatic activity than the amino acid sequence between Ile117 and Met234 [Sakamoto et al., 1999]. The data are consistent with another experiment [Hiratsuka et al., 1998] and the observation that the mutations identified in patients so far are located after the amino acid residue Arg183 (Table 1). Multiple cDNA clones were isolated from the human lymphocyte cDNA library [Leon-Del-Rio et al., 1995]. They differed from human liver cDNA mainly at their 5′ ends. Using an exon-trapping method, another type of cDNA was isolated from the human myeloid cell line KG-1 [Ohira et al., 1997]. This cDNA has the longest 5′ upstream and 3′ downstream untranslated regions. Because the first and second methionine codons found in other types of cDNAs were skipped, this cDNA should produce only the shortest form of HLCS protein [Hiratsuka et al., 1998].

Comparisons of sequences of multiple cDNAs and the 21q22.1 genomic region determined in the human genome project [Hattori et al., 2000] has elucidated the organization of the exons and introns of the human HLCS gene [Yang et al., 2001). The HLCS gene spans approximately 240 kb and comprises 14 exons (Fig. 1). The first methionine codon is located in exon 6, and a stop codon in exon 14. Of the 14 introns, intron 9 is the longest (about 130 kb). It contains two polymorphic tetra-nucleotide repeats that are useful for determining a haplotype of the HLCS gene [Yang et al., 2000]. On the basis of results reported for several cDNA species, the HLCS gene probably generates multiple transcripts, although the physiological significance of this is not yet clear. We demonstrated at least three mRNA types with different transcription starting sites in human cultured cells [Yang et al., 2001]. Type 1 mRNA starts at exon 1. Type 2 mRNA starts at exon 3 and type 3 mRNA starts at exon 2. The variations at the 5′ end of the mRNA so far identified do not change the amino acid sequence of HLCS. Substantial variations were also present in the 3′ untranslated region (UTR) sequence of HLCS, where no consensus sequence for the polyadenylation signal was found [Yang et al., 2001].

MUTATIONS AND POLYMORPHISMS

Mutations

During the last 10 years since the first report of the cDNA sequence [Suzuki et al., 1994], 30 mutations in the HLCS gene have been reported (Table 1). The table includes novel mutations identified in four Japanese, two Faroese patients, one Danish, and one Spanish patient whose cases were not reported previously. Mutations occur throughout the entire coding region except exons 6 and 10 (Fig. 1). The types of mutations are one single amino acid deletion, five single nucleotide insertions/deletions, 22 missense mutations, and two nonsense mutations. The only intronic mutation identified was a single nucleotide mutation in intron 10, c.1519+5G>A (also designated IVS10+5G>A), that causes a splice error [Sakamoto et al., 2000]. Two mutations, L237P and 780delG, account for 50% of the Japanese mutant alleles [Yang et al., 2001], but are never found in other ethnic groups. The IVS10+5G>A mutation was found in north European countries; details of this will be discussed in the next section. The mutations R508W, V550M, G581S, D571N, and R565X were found in several ethnic groups.

Polymorphisms

The current public SNP database (dbSNP) shows three SNPs in the coding region of HLCS. We identified two other polymorphisms during the investigation of patients with HLCS deficiency and their family members (Table 1).

CLINICAL RELEVANCE

Founder Mutations

The IVS10+5G>A mutation was first identified in a Swedish patient who showed her first symptoms at the age of 8 years. The amelioration of her biochemical and clinical abnormalities after biotin treatment started was relatively slow among patients with HLCS [Holme et al., 1988]. This mutation resulted in abnormal splicing with a decreased level of normal mRNA [Sakamoto et al., 2000]. Holocarboxylase synthetase activity in the patient's fibroblasts was 4% of the normal level [Sakamoto et al., 2000]. Subsequently, a Danish patient and two patients from the Faroe Islands who are homozygous for this mutation have been reported [Yang et al., 2001]. In addition, one French patient and one German patient were reported to have IVS10+5G>A in a heterozygous form [Santer et al., 2003; Yang et al., 2001]. We further analyzed the cases of two unrelated Faroese patients and another Danish patient and found that all of the patients were homozygous for the mutation; so far, 16 alleles have been identified (Table 1). To determine the origin of the mutations, we investigated polymorphic microsatellite markers in the *HLCS* gene and determined the haplotypes of patients [Yang et al., 2000]. All IVS10+5G>A alleles were associated with the 2-3 haplotype.

Geographically, the Faroe Islands are located in the North Atlantic, north of Scotland, and west of Norway [Ewald et al., 1999]. The Faroese immigrated from Norway about 1,000 years ago. They have expanded in number from a few thousand to about 47,000 during the last two centuries. Because we have already confirmed five patients from distinct families on this island, the incidence may be estimated to be at least 1 in 10,000. This value may be 10 times higher than those of countries in the rest of the world. These observations strongly suggest that IVS10+5G>A is a founder mutation in Scandinavian patients with HLCS deficiency [Yang et al., 2001]. In a Faroese family with an HLCS-deficient child, we found an asymptomatic father who was also homozygous for this mutation. He has never shown clinical symptoms throughout his life, suggesting that there may be other asymptomatic IVS10+5G>A homozygotes.

The mutations L237P and 780delG are predominant only in Japanese patients [Aoki et al., 1995; Sakamoto et al., 1998; Yang et al., 2000, 2001]. We investigated the haplotype of these two mutations and found that both mutant alleles were exclusively associated with haplotype 2-2. This finding is consistent with the notion that L237P and 780delG are founder mutations in the Japanese population [Yang et al., 2000]. Although patients were found who were homozygous for L237P or compound heterozygous for L237P and 780delG, none were found who were homozygous for 780delG, suggesting that homozygous 780delG mutations may be lethal.

TABLE 1. List of Mutations and Polymorphisms in the *HLCS* Gene*

Exon/intron	Nucleotide change in cDNA	Effect on coding region	Number of alleles	Patient origin	References
Mutations					
Exon 7	c.548G>C	p.R183R	2	Unknown	Sakamoto et al. [1999]
Exon 7	c.647T>G	p.L216R	3	Australian	Dupuis et al. [1996]
				Maori	Morrone et al. [2002]
Exon 7	c.655_656insA	p.I219NfsX58	2	Japanese	Yang et al. [2001]
Exon 7	c.710T>C	p.L237P	9	Japanese	Aoki et al. [1995]
					Yang et al. [2001]
Exon 7	c.780delG	p.G261VfsX20	7	Japanese	Aoki et al. [1995]
					Yang et al. [2001]
Exon 8	c.998T>A	p.V333E	1	German	Suormala et al. [1997]
					Aoki et al. [1999]
Exon 8	c.1053_1054insC	p.L353AfsX7	1	Spanish	Briones et al. [1989]
					Yang et al. [2001]
Exon 8	c.1080A>C	p.R360S	2	Japanese	Yang et al. [2001]
Exon 8	c.1088T>A	p.V363D	2	Unknown	Dupuis et al. [1996]
Exon 9	c.1367A>G	p.Y456C	1	Malaysian	Yang et al. [2001]
Exon 9	c.1385C>T	p.T462I	1	Spanish	Aoki et al. [1999]
					Yang et al. [2001]
Exon 9	c.1409T>C	p.L470S	1	Japanese	Yang et al. [2001]
Intron 10	c.1519+5G>A	Splice defect (IVS10+5G>A)	16	Spanish	Holme et al. [1988]
				Danish	Sakamoto et al. [2000]
				Faroese	Yang et al. [2001]
				Swedish	This study
				French	
				German	
Exon 11	c.1522C>T	p.R508W	15	Japanese	Dupuis et al. [1996]
				Taiwanese	Sakamoto et al. [1998]
				Chinese	Yang et al. [2001]
				Iranian	Morrone et al. [2002]
				Other	Tang et al. [2003]
Exon 11	c.1533T>A	p.N511K	1	Italian	Morrone et al. [2002]
Exon 11	c.1553G>A	p.G518E	1	Unknown	Dupuis et al. [1996]
Exon 11	c.1589delT	p.L529RfsX15	1	German	Aoki et al. [1999]
Exon 11	c.1640T>G	p.V547G	1	German	Yang et al. [2001]
Exon 11	c.1648G>A	p.V550M	8	Japanese	Dupuis et al. [1996]
				African	Aoki et al. [1997]
				Other	Morrone et al. [2002]; Tang et al. [2003]
Exon 12	c.1693C>T	p.R565X	2	Japanese	Sakamoto et al. [1998]
				African	Yang et al. [2001]
					Tang et al. [2003]
Exon 12	c.1711G>A	p.D571N	3	German	Aoki et al. [1999]
				Spanish	Dupuis et al. [1996]
					This study
Exon 12	c.1741G>A	p.G581S	4	Italian	Fuchshuber et al. [1993]
				Turkish	Suormala et al. [1997]
					Morrone et al. [2002]
Exon 12	c.1744G>A	p.G582R	1	Italian	Morrone et al. [2002]
Exon 13	c.1828_1830del	p.T610del	2	Lebanese	Aoki et al. [1999]
					Touma et al. [1999]
Exon 13	c.1843G>T	p.D615Y	1	Spanish	This study
Exon 13	c.1990G>T	p.D634Y	1	German	Suormala et al. [1997]
Exon 13	c.1990G>A	p.D634N	2	Japanese	This study
				Chinese	Tang et al. [2003]
Exon 13	c.1992delC	p.R665DfsX41	1	French	Suormala et al. [1997]
Exon 13	c.1993C>T	p.R665X	1	Japanese	This study
Exon 14	c.2144A>G	p.D715G	1	Japanese	This study
Polymorphisms of the coding region					
Exon 6	c.285C>T	p.P95P			dbSNP# rs2230182
Exon 7	c.126G>T	p.E42D		Malaysian	Yang et al. [2001]
Exon 7	c.843C>T	p.S278S			Aoki et al. [1999]
					dbSNP# rs1065758
Exon 7	c.971G>A	p.R324H		Spanish	This study
Exon 8	c.1053T>C	p.N351N			dbSNP# rs1065759

*Intronic sequence of c.1519+5G>A is based on AB063285; others are based on NT_000411.4 (A of ATG is +1).

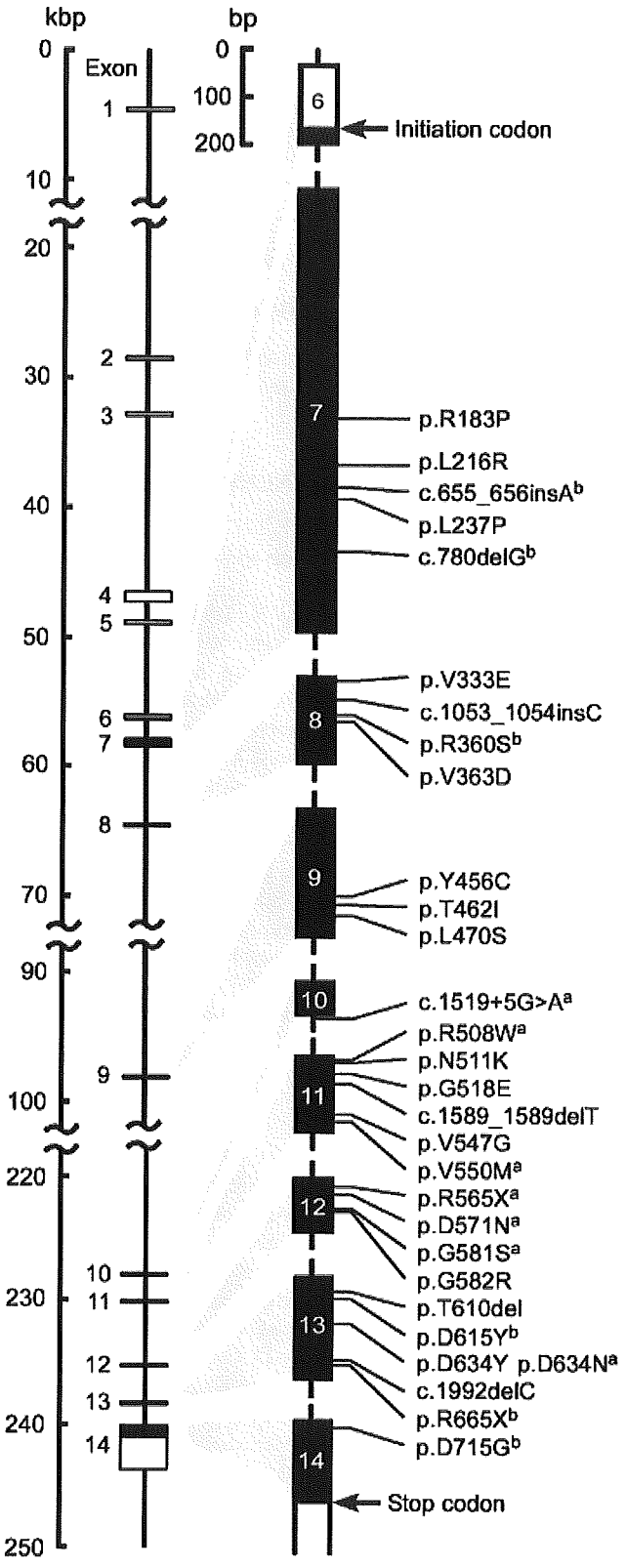

FIGURE 1. **Structure of the human holocarboxylase synthetase gene and a summary of its mutations. The *HLCS* gene spans approximately 240 kb and comprises 14 exons. The initiation codon is located in exon 6 and the stop codon is located in exon 14. Filled squares are coding exons. [a]Mutations found in several ethnic groups. [b]Mutations found in Japanese patients.**

Recurrent Mutations

The R508W mutation was found in a heterozygous form in three Japanese patients and one Chinese patient [Sakamoto et al., 1998; Tang et al., 2003; Yang et al., 2001] and in the homozygous form in three Chinese patients and one Iranian patient [Hwu et al., 2000; Morrone et al., 2002; Tang et al., 2003]. This mutation was also found in five mutant alleles in four U.S. patients whose ethnic backgrounds were not described [Dupuis et al., 1996]. The R508W mutation has been shown to have low activity in the cells [Dupuis et al., 1999]. Another mutation found in several ethnic groups is V550M. One Japanese patient was homozygous for this mutation [Aoki et al., 1997]. Dupuis et al. [1996] reported that three U.S. patients had this mutation (four alleles). Two Italian patients and one African patient were also reported to have the V550M mutation [Hwu et al., 2000; Morrone et al., 2002; Tang et al., 2003]. The mutation resulted in a decreased affinity of HLCS to biotin (i.e., a Km mutant); the mutant HLCS shows its enzymatic activity to some extent at high biotin concentrations [Aoki et al., 1997]. The R508W mutant alleles in Japanese patients were associated with either haplotype 2-3 or haplotype 1-4 [Yang et al., 2000]. The homozygous R508W mutation in the Taiwanese patient was associated with haplotype 2-3. The haplotype of the V550M mutation in the Japanese patient was 1-4, whereas that in the Jewish patient was 2-3. The nucleotide change in the R508W and the V550M mutations is a C-to-T transition at the CpG dinucleotides. These data suggest that R508W and V550M are not founder mutations but recurrent mutations occurring at CpG dinucleotide mutation hotspots [Yang et al., 2000].

Genotype–Phenotype Correlation

From an early kinetic study of mutant HLCS, patients were thought to be responsive to biotin treatment because they have mutant HLCS with a decreased affinity for biotin (i.e., a Km mutant) [Burri et al., 1985]. Using a transient expression system in HLCS-deficient fibroblasts, we further investigated the enzymatic characters of mutant enzymes [Aoki et al., 1997; Sakamoto et al., 1999]. The enzymes containing mutations that are located outside the biotin-binding region (R183P, L216R, L237P, V333E, and V363D) showed normal to higher affinity for biotin but decreased Vmax (i.e., they were non-Km mutants). The mutations located in the biotin-binding region (Gly581Ser and Thr610del) showed lower affinity for biotin (i.e., Km mutants). We usually expect that a "Km mutant" recovers its activity with high biotin concentration but a "non-Km mutant" does not. However, patients with non-Km mutants responded clinically to pharmacological doses of biotin in a manner similar to that of patients with Km mutants. The observation may be explained by our experimental data that the Km for biotin of normal HLCS is higher than the concentration of biotin in human cells under ordinary nutritional conditions [Aoki et al., 1997; Sakamoto et al., 1999].

In 16 Japanese patients from apparently unrelated families whose clinical pictures were well known, nine patients developed symptoms within a week after birth. Eight of these were compound heterozygotes of a point mutation and a null mutation (insertion/deletion and nonsense mutations). The point mutations were L237P and L470S. The null mutations were 780delG, 6556insA, and R665X. Only one patient was homozygous for L237P. We studied mutations in two European patients who displayed symptoms before they were 1 week old. One was found to be a compound heterozygote of T462I and 1053_1054insC and the other was found to be a homozygote of G581S [Yang et al., 2001].

The expression study suggested that mutant HLCS with T462I or G581S showed less than 5% of the normal activity [Aoki et al., 1999]. We never found HLCS-deficient patients who harbor two null mutations. From these observations, we can speculate that homozygous null mutations are lethal in utero and combination of a null mutation and a point mutation that shows less than a few percent of the normal activity results in neonatal onset.

In eight clinically well-documented patients who have at least one allele of R508W, none displayed symptoms before the age of 2 months. Patients are biotin responsive and the prognosis was fine except in one case [Morrone et al., 2002]. This mutant enzyme's favorable response to biotin was also demonstrated by in vitro experiments [Dupuis et al., 1999]. Although the number of cases to study is still limited, patients with V550M are also expected to show good prognosis [Aoki et al., 1997; Morrone et al., 2002]. The IVS10+5G>A mutation shows a unique clinical character. Onset of the homozygotes of this mutation ranges from 2 months to 8 years (median 5 months) [Santer et al., 2003; Yang et al., 2001]. As previously noted, some patients homozygous for this mutation may be asymptomatic. In spite of a rather mild phenotype associated with IVS10+5G>A among HLCS-deficient patients at the onset, the clinical responses to biotin treatment are slow and partial in some cases [Holme et al., 1988; Santer et al., 2003].

In conclusion, there is a relationship between clinical biotin responsiveness and the residual activity of HLCS. We can predict the response to biotin therapy from genotypes in a patient who has a combination of well-characterized mutations stated above. However, we should be aware that some variation of outcome exists between patients with the same mutation.

DIAGNOSTIC RELEVANCE

Clinical pictures of HLCS deficiency and those of biotinidase deficiency are often indistinguishable. It is essential to perform enzymatic or DNA assays to identify the primary defect of patients who show MCD. Out of 16 HLCS-deficient Japanese patients from independent families, 15 have at least one allele of the four major mutations (del780C, L237P, R508W, and V550M). We routinely screen for these four mutations in our laboratory as the initial step for diagnosis of MCD in Japanese patients. If none of the four mutations are identified, we perform an enzyme assay [Suzuki et al., 1996]. In patients from ethnic groups other than the Faroese, mutations appear to be diverse. Thus, an enzyme assay is the first choice for the definitive diagnosis of HLCS deficiency in many ethnic groups. In the Faroese, DNA examination to detect IVS10+5G>T is useful.

FUTURE PROSPECTS

Information on HLCS mutations is still limited and further investigation into the mutation spectrum of each ethnic group is required to facilitate the diagnostic DNA examination of patients with MCD.

As exemplified by the recent works, roles of HLCS other than the biotinylation of carboxylases may exist [Narang et al., 2004; Solorzano-Vargas et al., 2002]. Newly identified functions of HLCS may be responsible for various symptoms observed in patients with HLCS deficiency.

Recently, a patient with MCD and a deficiency of the biotin transporter in the peripheral blood cells was reported [Mardach et al., 2002]. No abnormalities were found in biotinidase or HLCS, indicating that MCD can be caused by defects in three different primary substances: HLCS, biotinidase, and biotin transporter. We encountered patients who showed biotin-responsive MCD symptoms with normal HLCS and biotinidase activity (unpublished results). The *SMVT* gene coding for biotin transport had been investigated as a candidate disease-causing gene. However, the reported patient and our patients had no mutation in the gene (unpublished results) [Mardach et al., 2002]. Identification of the third gene that causes MCD remains to be elucidated in the field of human biotin metabolism.

ACKNOWLEDGMENTS

We thank the physicians who referred patients with HLCS deficiency. We also thank Kumi Kato for the excellent technical assistance.

REFERENCES

Achuta Murthy PN, Mistry SP. 1972. Synthesis of biotin-dependent carboxylases from their apoprotein and biotin. J Sci Ind Res (India) 31:554–563.

Aoki Y, Suzuki Y, Sakamoto O, Li X, Takahashi K, Ohtake A, Sakuta R, Ohura T, Miyabayashi S, Narisawa K. 1995. Molecular analysis of holocarboxylase synthetase deficiency: a missense mutation and a single base deletion are predominant in Japanese patients. Biochim Biophys Acta 1272:168–174.

Aoki Y, Suzuki Y, Li X, Sakamoto O, Chikaoka H, Takita S, Narisawa K. 1997. Characterization of mutant holocarboxylase synthetase (HCS): a Km for biotin was not elevated in a patient with HCS deficiency. Pediatr Res 42:849–854.

Aoki Y, Li X, Sakamoto O, Hiratsuka M, Akaishi H, Xu L, Briones P, Suormala T, Baumgartner ER, Suzuki Y, Narisawa K. 1999. Identification and characterization of mutations in patients with holocarboxylase synthetase deficiency. Hum Genet 104:143–148.

Baumgartner ER, Suormala T. 1997. Multiple carboxylase deficiency: inherited and acquired disorders of biotin metabolism. Int J Vitam Nutr Res 67:377–384.

Briones P, Ribes A, Vilaseca MA, Rodriguez-Valcarcel G, Thuy LP, Sweetman L. 1989. A new case of holocarboxylase deficiency. J Inherit Metab Dis 12:329–330.

Burri B, Sweetman L, Nyhan WL. 1985. Heterogeneity of holocarboxylase synthetase in patients with biotin-responsive multiple carboxylase deficiency. Am J Hum Genet 37:326–337.

Chikaoka H, Nagano M, Takita S, Shinka T, Kuhara T, Inoue Y, Matsumoto I. 1992. Multiple carboxylase deficiency: a case report. In: Matsumoto I, editor. Advances in chemical diagnosis and treatment of metabolic diseases. New York: John Willy & Sons. p 31–36.

Dupuis L, Leon-Del-Rio A, Leclerc D, Campeau E, Sweetman L, Saudubray JM, Herman G, Gibson KM, Gravel RA. 1996. Clustering of mutations in the biotin-binding region of holocarboxylase synthetase in biotin-responsive multiple carboxylase deficiency. Hum Mol Genet 5:1011–1016.

Dupuis L, Campeau E, Leclerc D, Gravel RA. 1999. Mechanism of biotin responsiveness in biotin-responsive multiple carboxylase deficiency. Mol Genet Metab 66:80–90.

Ewald H, Wang AG, Vang M, Mors O, Nyegaard M, Kruse TA. 1999. A haplotype-based study of lithium responding patients with bipolar affective disorder on the Faroe Islands. Psychiatr Genet 9:23–34.

Fuchshuber A, Suormala T, Roth B, Duran M, Michalk D, Baumgartner ER. 1993. Holocarboxylase synthetase deficiency: early diagnosis and management of a new case. Eur J Pediatr 152:446–449.

Gibson KM, Bennett MJ, Nyhan WL, Mize CE. 1996. Late-onset holocarboxylase synthetase deficiency. J Inherit Metab Dis 19:739–742.

Hattori M, Fujiyama A, Taylor TD, Watanabe H, Yada T, Park HS, Toyoda A, Ishii K, Totoki Y, Choi DK, Soeda E, Ohki M, Takagi T, Sakaki Y, Taudien S, Blechschmidt K, Polley A, Menzel U, Delabar J, Kumpf K, Lehmann R, Patterson D, Reichwald K, Rump A, Schillhabel M, Schudy A. 2000. The DNA sequence of human chromosome 21. The chromosome 21 mapping and sequencing consortium. Nature 405:311–319.

Hiratsuka M, Sakamoto O, Li X, Suzuki Y, Aoki Y, Narisawa K. 1998. Identification of holocarboxylase synthetase (HCS) proteins in human placenta. Biochim Biophys Acta 1385: 165–171.

Holme E, Jacobson CE, Kristiansson B. 1988. Biotin-responsive multiple carboxylase deficiency in an 8-year-old boy with normal serum biotinidase and fibroblast holocarboxylase-synthetase activities. J Inherit Metab Dis 11:270–276.

Hwu W, Suzuki Y, Yang X, Li X, Chou S, Narisawa K, Tsai W. 2000. Late-onset holocarboxylase synthetase deficiency with homologous R508W mutation. J Formos Med Assoc 99:174–177.

Leon-Del-Rio A, Leclerc D, Akerman B, Wakamatsu N, Gravel RA. 1995. Isolation of a cDNA encoding human holocarboxylase synthetase by functional complementation of a biotin auxotroph of Escherichia coli. Proc Natl Acad Sci USA 92: 4626–4630.

Mardach R, Zempleni J, Wolf B, Cannon MJ, Jennings ML, Cress S, Boylan J, Roth S, Cederbaum S, Mock DM. 2002. Biotin dependency due to a defect in biotin transport. J Clin Invest 109:1617–1623.

Morrone A, Malvagia S, Donati MA, Funghini S, Ciani F, Pela I, Boneh A, Peters H, Pasquini E, Zammarchi E. 2002. Clinical findings and biochemical and molecular analysis of four patients with holocarboxylase synthetase deficiency. Am J Med Genet 111:10–18.

Narang MA, Dumas R, Ayer LM, Gravel RA. 2004. Reduced histone biotinylation in multiple carboxylase deficiency patients: a nuclear role for holocarboxylase synthetase. Hum Mol Genet 13:15–23.

Narisawa K, Arai N, Igarashi Y, Satoh T, Tada K. 1982. Clinical and biochemical findings on a child with multiple biotin-responsive carboxylase deficiencies. J Inher Metab Dis 5:67–68.

Ohira M, Seki N, Nagase T, Suzuki E, Nomura N, Ohara O, Hattori M, Sakaki Y, Eki T, Murakami Y, and others. 1997. Gene identification in 1.6-Mb region of the Down syndrome region on chromosome 21. Genome Res 7:47–58.

Pacheco-Alvarez D, Solorzano-Vargas RS, Del Rio AL. 2002. Biotin in metabolism and its relationship to human disease. Arch Med Res 33:439–447.

Peters DM, Griffin JB, Stanley JS, Beck MM, Zempleni J. 2002. Exposure to UV light causes increased biotinylation of histones in Jurkat cells. Am J Physiol Cell Physiol 283:C878–C884.

Sakamoto O, Suzuki Y, Aoki Y, Li X, Hiratsuka M, Yanagihara K, Inui K, Okabe T, Yamaguchi S, Kudoh J, Shimizu N, Narisawa K. 1998. Molecular analysis of new Japanese patients with holocarboxylase synthetase deficiency. J Inherit Metab Dis 21:873–874.

Sakamoto O, Suzuki Y, Li X, Aoki Y, Hiratsuka M, Suormala T, Baumgartner ER, Gibson KM, Narisawa K. 1999. Relationship between kinetic properties of mutant enzyme and biochemical and clinical responsiveness to biotin in holocarboxylase synthetase deficiency. Pediatr Res 46:671–676.

Sakamoto O, Suzuki Y, Li X, Aoki Y, Hiratsuka M, Holme E, Kudoh J, Shimizu N, Narisawa K. 2000. Diagnosis and molecular analysis of an atypical case of holocarboxylase synthetase deficiency. Eur J Pediatr 159:18–22.

Santer R, Muhle H, Suormala T, Baumgartner ER, Duran M, Yang X, Aoki Y, Suzuki Y, Stephani U. 2003. Partial response to biotin therapy in a patient with holocarboxylase synthetase deficiency: clinical, biochemical, and molecular genetic aspects. Mol Genet Metab 79:160–166.

Solorzano-Vargas RS, Pacheco-Alvarez D, Leon-Del-Rio A. 2002. Holocarboxylase synthetase is an obligate participant in biotin-mediated regulation of its own expression and of biotin-dependent carboxylases mRNA levels in human cells. Proc Natl Acad Sci USA 99:5325–5330.

Stanley JS, Griffin JB, Zempleni J. 2001. Biotinylation of histones in human cells. Effects of cell proliferation. Eur J Biochem 268:5424–5429.

Suormala T, Fowler B, Duran M, Burtscher A, Fuchshuber A, Tratzmuller R, Lenze MJ, Raab K, Baur B, Wick H, Baumgartner ER. 1997. Five patients with a biotin-responsive defect in holocarboxylase formation: evaluation of responsiveness to biotin therapy in vivo and comparative biochemical studies in vitro. Pediatr Res 41:666–673.

Suormala T, Fowler B, Jakobs C, Duran M, Lehnert W, Raab K, Wick H, Baumgartner ER. 1998. Late-onset holocarboxylase synthetase-deficiency: pre- and post-natal diagnosis and evaluation of effectiveness of antenatal biotin therapy. Eur J Pediatr 157:570–575.

Suzuki Y, Aoki Y, Ishida Y, Chiba Y, Iwamatsu A, Kishino T, Niikawa N, Matsubara Y, Narisawa K. 1994. Isolation and characterization of mutations in the human holocarboxylase synthetase cDNA. Nat Genet 8:122–128.

Suzuki Y, Aoki Y, Sakamoto O, Li X, Miyabayashi S, Kazuta Y, Kondo H, Narisawa K. 1996. Enzymatic diagnosis of holocarboxylase synthetase deficiency using apo-carboxyl carrier protein as a substrate. Clin Chim Acta 251:41–52.

Tang NL, Hui J, Yong CK, Wong LT, Applegarth DA, Vallance HD, Law LK, Fung SL, Mak TW, Sung YM, Cheung KL, Fok TF. 2003. A genomic approach to mutation analysis of holocarboxylase synthetase gene in three Chinese patients with late-onset holocarboxylase synthetase deficiency. Clin Biochem 36: 145–149.

Touma E, Suormala T, Baumgartner ER, Gerbaka B, Ogier de Baulny H, Loiselet J. 1999. Holocarboxylase synthetase deficiency: report of a case with onset in late infancy. J Inherit Metab Dis 22:115–122.

Wolf B, Hsia YE, Sweetman L, Nyhan WL. 1981. Multiple carboxylase deficiency: clinical and biochemical improvement following neonatal biotin treatment. Pediatrics 68:113–118.

Wolf B. 2001. Disorders of biotin metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular basis of inherited disease. New York: McGraw-Hill. p 3935–3962.

Yang X, Aoki Y, Li X, Sakamoto O, Hiratsuka M, Gibson KM, Kure S, Narisawa K, Matsubara Y, Suzuki Y. 2000. Haplotype analysis suggests that the two predominant mutations in Japanese patients with holocarboxylase synthetase deficiency are founder mutations. J Hum Genet 45:358–362.

Yang X, Aoki Y, Li X, Sakamoto O, Hiratsuka M, Kure S, Taheri S, Christensen E, Inui K, Kubota M, Ohira M, Ohki M, Kudoh J, Kawasaki K, Shibuya K, Shintani A, Asakawa S, Minoshima S, Shimizu N, Narisawa K, Matsubra Y, Suzuki Y. 2001. Structure of human holocarboxylase synthetase gene and mutation spectrum of holocarboxylase synthetase deficiency. Hum Genet 109:526–534.