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Case Report

Niemann-Pick Disease Type C: Cataplexy and Hypocretin in Cerebrospinal Fluid

KATSUYUKI OYAMA, TSUTOMU TAKAHASHI, YUTAKA SHOJI, MIKA OYAMADA, ATSUKO NOGUCHI, HIROAKI TAMURA, GORO TAKADA and TAKASHI KANBAYASHI[1]

Departments of Pediatrics and [1]Psychiatry, Akita University School of Medicine, Akita, Japan

OYAMA, K., TAKAHASHI, T., SHOJI, Y., OYAMADA, M., NOGUCHI, A., TAMURA, H., TAKADA, G. and KANBAYASHI, T. *Niemann-Pick Disease Type C: Cataplexy and Hypocretin in Cerebrospinal Fluid*. Tohoku J. Exp. Med., 2006, **209** (3), 263-267 —— Niemann-Pick disease type C (NPC) is an inherited lipid storage disorder, characterized by a defect in intracellular trafficking of exogenous cholesterol that leads to the lysosomal accumulation of unesterified cholesterol. We report a Japanese patient with NPC caused by a homozygous c.2974 G > T mutation of the *NPC1* gene, which predicts a glycine (GGG) to tryptophan (TGG) change at codon 992 (designated as p.G992W). This is a well-known *NPC1* gene mutation that causes a unique phenotype of NPC, which has been limited to a single Acadian ancestor in Nova Scotia, Canada. Our patient characteristically started presenting with cataplexy at the age of 9 years. Recent studies have shown reduced hypocretin-1 levels in the cerebrospinal fluid (CSF) of narcoleptic patients with cataplexy. In our patient, the level of hypocretin-1 was determined as moderately low, 174 pg/ml (normal, > 200 pg/ml). To date, CSF levels of hypocretin-1 have been determined by using an identical assay method in 7 cases of NPC, including our case. All of the NPC cases with cataplexy demonstrated low levels of CSF hypocretin-1, confirming the association of reduced CSF hypocretin-1 levels with cataplexy in NPC. ———— Niemann-Pick disease type C; NPC1 gene; Nova Scotia; cataplexy; hypocretin-1

Niemann-Pick disease type C (NPC) (MIM 257220 and MIM 601015) is an inherited lipid storage disorder, characterized by a defect in intracellular trafficking of exogenous cholesterol that leads to the lysosomal accumulation of unesterified cholesterol, which can be demonstrated by filipin staining in cultured NPC cells (Patterson et al. 2001). Two genetic complementation groups, NPC1 and NPC2, have been identified as the genetic defect in this disease and *NPC1* is the responsible gene for 90 - 95% of NPC cases (Steinberg et al. 1994; Vanier et al. 1996; Carstea et al. 1997; Naureckiene et al. 2000). The patients typically manifest hepatosplenomegaly and progressive neurological symptoms including vertical supranuclear ophthalmoplegia, progressive ataxia, dystonia, and dementia, with varying age at onset. Interestingly, several cases of NPC with cataplexy have been reported (Kandt et al. 1982; Denoix et al. 1991; Challamel et al. 1994; Boor and Reitter

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Correspondence: Tsutomu Takahashi, Department of Pediatrics, Akita University School of Medicine, 1-1-1, Hondo, Akita 010-8543, Japan.
e-mail: tomy@med.akita-u.ac.jp

1997). In cataplexy, sudden and brief episodes of bilateral loss of muscle tone are observed during strong emotions such as laughing or anger. Narcolepsy, a chronic sleep disorder, is characterized by excessive daytime sleep, cataplexy, and other abnormal manifestations of rapid eye movement sleep. Recent studies showed reduced hypocretin-1 levels in the cerebrospinal fluid (CSF) of narcoleptic patients with cataplexy (Nishino and Kanbayashi 2005). In contrast, most narcolepsy or hypersomnia cases without cataplexy have normal CSF hypocretin-1 levels (Nishino and Kanbayashi 2005). Two recent articles reported moderately low levels of hypocretin-1 in the CSF of NPC patients with cataplexy (Kanbayashi et al. 2003; Vankova et al. 2003).

Niemann-Pick disease type D (NPD) had been known as a variant of NPC, characterized by mild to moderate hepatosplenomegaly, sea-blue histiocytes in the bone marrow, supranuclear gaze paresis in the vertical plane, slowly progressing ataxia, and mental retardation (Jan and Camfield 1998; Patterson et al. 2001). Patients with NPD have been traced to a single Acadian ancestor in Nova Scotia (Canada). However, analyses of the *NPC1* gene in patients with NPD identified a point mutation within this gene (c.2974 G > T, or p.G 992 W) that shows complete linkage disequilibrium with NPD, confirming that NPD is an allelic variant of NPC (Greer et al. 1998). In this report, we describe a Japanese patient with NPC caused by a homozygous c.2974 G > T mutation of the *NPC1* gene, or an NPD-causing mutation. The patient characteristically started presenting with cataplexy at the age of 9 years. The level of hypocretin-1 was determined as moderately low in the CSF. We here discuss the relation of cataplexy with levels of CSF hypocretin-1 in the patients with NPC.

Case Report

The patient was the daughter of consanguineous parents, who were second cousins. After 40 weeks of pregnancy, she was uneventfully born with birth weight 2,414 g (-S.D.) and birth length 47 cm (-S.D.). At the age of 1 month, the patient showed mild hepatosplenomegaly and mild liver dysfunction, but these did not become symptomatic during infancy. She normally developed until the age of 4 years. At the age of 5 years, the patient started presenting with walking disability. At the age of 8.5 years, she was referred to our hospital for clinical evaluation of ataxic gait. Height and weight were 131.5 cm (+0.7 S.D.) and 39.6 kg (+2.7 S.D.), respectively. The patient showed mild hepatomegaly, but there were no other clinical findings, including characteristic face, splenomegaly, and bone deformity, suggesting some type of storage disease. Neurological examinations were not outstanding, but mild atrophy of the cerebrum was bilaterally observed on brain magnetic resonance imaging (MRI) study. Screening tests for metabolic diseases, including serum and urinary amino acids, urinary organic acids, urinary uronic acids, lactate and pyruvate, and sialic acid, were all negative. Cataplexy was apparent at the age of 9.0 years and became frequent during the day. Cataplexy demonstrated typical attacks, triggered by strong emotions such as laughing or anger, leading to bilateral loss of general muscle tone. At the age of 10 years, she showed supranuclear gaze paresis in the vertical plane. These neurological findings led to a diagnosis of NPC in the patient.

First, lysosomal acid sphingomyelinase activity, which is defective in Niemann-Pick disease types A and B, was determined as normal. For the diagnosis of NPC, storage of free cholesterol was examined by filipin staining of fibroblast cells from the patient. The filipin staining is based on the reaction of unesterified cholesterol with fluorescent antibiotic filipin giving a strongly fluorescent and stable cholesterol-filipin complex suitable for in situ detection. The fibroblasts were maintained in the medium supplemented with 10% fetal bovine serum. For filipin staining, fibroblast cells from the patient and normal control were stained with 300 μg/ml of filipin complex (Sigma-Aldrich Corporation, St. Louis, MO, USA) after fixation with 2% paraformaldehyde. As a positive control, fibroblast cell from a patient with the severe phenotype of NPC due to a homozygous *NPC1* gene mutation of c.3615delA was also stained with filipin (Tamura et al. 2006).

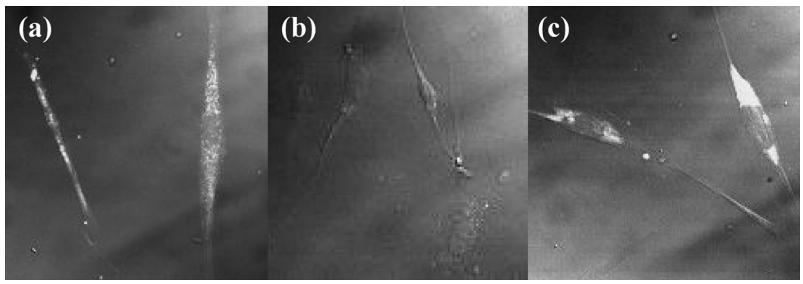

Fig. 1. Filipin staining of fibroblast cells. Fibroblast cells from the patient (a) were positively stained with filipin in contrast to the negative staining in normal control (b), but the level of fluorescence was significantly lower in comparison with the severe phenotype of NPC (c).

Stained cells were examined using a Zeiss LSM 510 META confocal microscope (Carl Zeiss, Thornwood, NY, USA) equipped with UV laser. Compared to normal fibroblasts, fibroblast cells from the patient had intracellular distributions of cholesterol with filipin staining in a characteristic distribution near the nucleus (Fig. 1). However, compared to the severe phenotype, the level of fluorescence was significantly lower.

For genetic analysis, blood sample was obtained from the patient after obtaining informed consent from the parents. The analysis was conducted following the method described before (Tamura et al. 2006). A homozygous c.2974G > T mutation, which predicts a glycine (GGG) to tryptophan (TGG) change at codon 992 (designated as p.G 992 W), was identified in the sequence of the *NPC1* gene from the patient (Fig. 2). We could not analyze the *NPC1* genes from the parents.

The CSF hypocretin-1 was quantified by ^{124}Iodine Hcrt-1 radioimmunoassay (Phoenix Pharmaceuticals, Belmont, CA, USA) (Kanbayashi et al. 2002). The level of CSF hypocretin-1 in this patient was determined to be moderately low, 174 pg/ml.

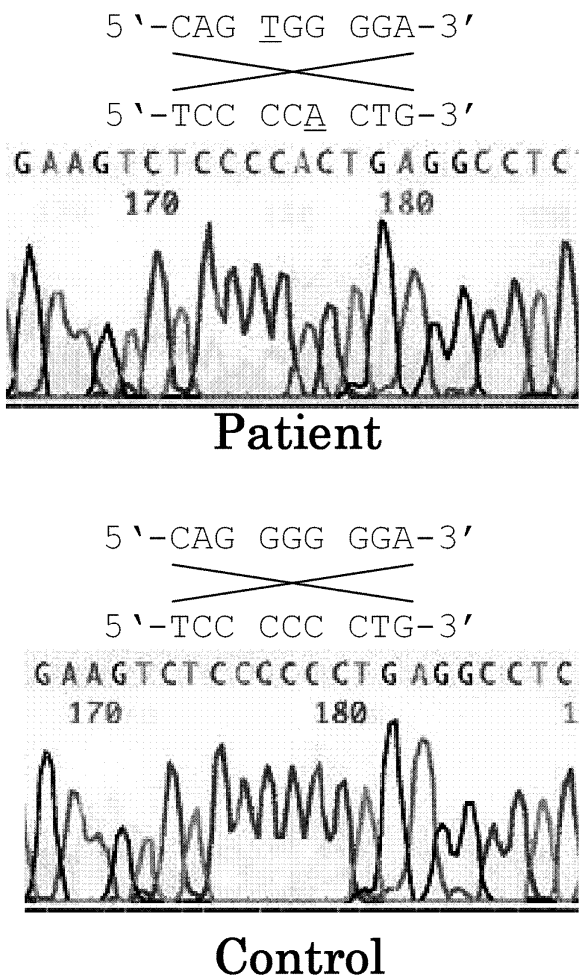

Fig. 2. Nucleotide sequence of *NPC1* gene mutation identified in the patient. The patient had a homozygous G-to-T transition at nucleotide 2,974 of the *NPC1* cDNA, which predicted a glycine (GGG) to tryptophan (TGG) change at codon 992 (designated as p.G 992 W).

Discussion

We reported a patient with NPC who characteristically presented with cataplexy. The genetic mutation, c.2974 G > T, identified from the patient is a well-known molecular variant of the *NPC1* gene that causes a variant of NPC, initially described as NPD, and was specifically derived from a single Acadian ancestor in Nova Scotia

(Canada) (Greer et al. 1998). In this variant, early milestones are normal in the majority of patients (Jan and Camfield 1998). Neurologic symptoms generally develop between 5 and 10 years of age with a mean age of 7.2 years at diagnosis. Seizures develop in all patients between 4.5 and 16 years of age, followed by significant physical and mental deterioration. Age at death ranges between 11 and 22.5 years. Our patient showed the same course as typical of patients with NPD from Nova Scotia. This is the first case of NPD due to a homozygous c.2974 G > T *NPC1* mutation found in another ethnic group, unrelated to Nova Scotia. Thus, our case suggested that ethnic background does not apparently influence the genotype-phenotype correlations observed in NPD.

It was reported that cataplectic attacks were observed in more than 10% of NPC cases (Vanier 1983). However, cataplexy was not described as a clinical feature in 20 patients with NPD (Jan and Camfield 1998). Cataplexy may not be a symptom particularly found in the patients with NPD. Our patient characteristically started presenting with cataplexy at the age of 9 years. The level of CSF hypocretin-1 was determined as moderately low, 174 pg/ml, in the patient. To date, levels of CSF hypocretin-1 have been determined in 7 cases of NPC, including our case (Table 1) (Kanbayashi et al. 2003; Vankova et al. 2003). Although the 7 samples were assayed at 2 different facilities, an identical assay method, ^{124}Iodine Hcrt-1 radioimmunoassay, was used for the measurements. Therefore, these data were considered comparable. CSF hypocretin-1 levels are normally above 200 pg/ml regardless of gender, age (from neonatal to 70S) and time of the CSF collections (Kanbayashi et al. 2002). Using the assay method described above, CSF hypocretin-1 levels are defined as low (< 110 pg/ml), intermediate (110 - 200 pg/ml) and normal (> 200 pg/ml) (Nishino and Kanbayashi 2005).

Of the 7 patients with NPC (Table 1), 4 patients (cases 1, 4, 6, and 7) showed reduced levels of CSF hypocretin-1, defined as intermediate low (110 - 200 pg/ml). Three of these patients, cases 1, 6, and 7, presented with cataplexy during their clinical course, suggesting the association of reduced CSF hypocretin-1 levels with cataplexy in NPC. A low CSF hypocretin-1 level was found in case 4, but no cataplexy was noticed in this subject (Vankova et al. 2003). This case suggests that the finding of low CSF hypocretin-1 may not be sufficient for clinical manifestation of cataplexy. Vankova et al. (2003) hypothesized that human leukocyte antigen positivity, NPC1 mutation, and hypocretin-1 deficiency all contribute to the clinical picture with regard to the presence of cataplexy. Case 5 had a severe phenotype caused by a homozygous truncation mutation. This patient did not show cataplexy during her entire clinical course, nor reduced CSF hypocretin-1 level, indicating that the reduced CSF hypocretin-1 level does not correspond to the severity of neurological phenotype. This reduction of CSF hypocretin-1 level might be caused by dysfunction of hypocretin-1 containing cells in

TABLE 1. Levels of CSF hypocretin-1 in the cases of Niemann-Pick disease type C.

	Cataplexy	Hypocretin-1 (> 200 pg/ml)	Genotype	Clinical features
Case 1[1)]	+	176		female, 14 yr
Case 2[1)]	–	297		female, 25 yr
Case 3[1)]	–	348		male, 24 yr
Case 4[1)]	–	143		male, 31 yr
Case 5[2)]	–	299	c.3615delA	severe, die at 4 yr old
Case 6[2)]	+	178		male, 4 yr
Case 7[3)]	+	174	c.2974G > T	Nova Scotia type

[1)]Vankova et al. (2003), [2)]Kanbayashi et al. (2003), [3)]this case.

the lateral hypothalamus due to intracellular cholesterol dysregulation. However, the dyfunction of hypocretin-1 containing cells, reducing the CSF hypocretin-1 level, does not stem from the severe neuronal damage observed in the NPC patient with severe phenotype.

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