Table 2. Relationship between the pteridine profile, the PKU profile, and TPN

Case	Age at urine sampling	Neopterin	Biopterin	N/B ratio	PKU profile	TPN
Case 1						
1st	4Y10M	2.35	1.09	2.16	–	–
2nd	8Y3M	38.08	1.13	33.72	+	+
Case 2						
1st	11Y	4.45	6.05	0.74	+	+
2nd	11Y2M	0.88	1.58	0.55	–	–
Control	5–10Y	0.59 ± 0.29	1.31 ± 0.58	0.5 ± 0.2	–	–
	10–16Y	0.43 ± 0.25	0.96 ± 0.41	0.5 ± 0.1	–	–

Values for pteridine are expressed as mmol/mol creatinine. Y, year; M, month.
The 1st and 2nd analyses for both cases were of the same urine specimens used for the metabolic profiling shown in Table 1.

disorders characterized by hyperphenylalaninemia and a deficiency of monoamine neurotransmitters. BH4 deficiency is caused by mutations in the genes encoding six enzymes responsible for BH4 biosynthesis and regeneration.[10]

In contrast to classical forms of BH4 deficiency, two others, DOPA-responsive dystonia, caused by autosomal dominant mutations in the GTP cyclohydrolase I gene, and sepiapterin reductase deficiency, an autosomal recessive disease, do not present with hyperphenylalaninemia and thus cannot be detected by neonatal screening for PKU.[11] Phe loading, however, can unmask these disorders by causing hyperphenylalaninemia. The amount of Phe supplied by TPN per day corresponds to that used in the standard Phe loading test. Neonates on TPN can show transient PKU profiles,[12] but older patients usually do not, unless there is additional underlying factor at play. Patients with DOPA-responsive dystonia or sepiapterin reductase deficiency, however, have such a factor, and have a high risk of developing hyperphenylalaninemia during TPN.

The concentrations of neopterin and biopterin in the present cases were determined by a method reported previously[13] and are shown in Table 2. Neither patient had dystonia nor a low neopterin level (Table 2). BH4 biosynthesis is stimulated by Phe and suppressed by BH4. The metabolic profiles taken when the patients were off TPN showed a normal pterin profile in case 2 and a normal biopterin profile in case 1, with a slight increase in neopterin that might have been caused by the infectious pneumonia. When the patients were on TPN, the metabolic profile in case 2 showed significantly increased neopterin and biopterin, with a normal neopterin/biopterin ratio, and that in case 1 showed markedly increased neopterin with a very high neopterin/biopterin ratio. This marked increase in neopterin also indicated that GTP cyclohydrolase I was not defective in case 1.

Recent studies on BH4 uptake demonstrate that the liver can take up BH4 only indirectly, via a pathway involving prior oxidation and dihydrofolate reductase reaction, and that BH4 deposition in the liver is completely inhibited by prior treatment with methotrexate, a folate analogue and dihydrofolate reductase inhibitor.[14,15] Methotrexate also inhibits the PAH system by preventing the regeneration of BH4 as a dihydropteridine reductase inhibitor.[16,17] Hyperphenylalaninemia and acute or subacute neurological disorders have been occasionally reported in patients receiving high-dose methotrexate therapy.[16,18] Although a large amount of folinic acid was administered after methotrexate chemotherapy to rescue the tetrahydrofolate and folate metabolism, this treatment may not rescue the BH4 metabolism.

In the urine from case 2 during TPN and after methotrexate chemotherapy, an unknown metabolite was detected having GC retention time of 13.455 min. The highest mass was detected at m/z 525 and the base peak at m/z 409 [M–116]. Ions at m/z 73, 117, 147, 408 [M–117] and 510 [M–15] are found suggesting biopterin-4TMS. The mass spectrum and the retention time were identical with those of authentic biopterin. The presence of biopterin suggested that q-dihydrobiopterin, the substrate for dihydropteridine reductase, was increased. It was, however, suggested that for the quantitative evaluation of these pterins using GC/MS, analysis at higher column temperature should be performed. Two other unknown peaks are under structural elucidation.

In general, the severity of the adverse effects caused by metabolic mimics of PKU may depend on the age of the patients and the duration of the metabolic profile. TPN was used for only a short time in the present cases, and the PKU profile cannot be attributed solely to the TPN. The time difference between the end of the infusion and the collection of the urine specimens was not recorded for either case. It is not clear, therefore, whether the PKU profiles we observed were representative, or if the profile was at times more severe. Nevertheless, the present study suggests that urine metabolic profiling is useful for understanding the metabolic state of patients receiving TPN during various medical treatments. It is important to determine the optimal nutritional formula, not only for neonates but also for younger children who undergo methotrexate chemotherapy, especially when TPN must be provided for a long period of time. If a PKU profile is detected during methotrexate chemotherapy, the administration of BH4 or lowering of the Phe content in the TPN may be effective.

CONCLUSIONS

We found that two patients had metabolic profiles characteristic of PKU while receiving TPN, but normal profiles without TPN. Although the marginally high Phe content of the TPN was almost certainly not the sole cause of these results except for neonates, its effect, combined with other factors including genetic factors and drugs such as methotrexate, led to the PKU profile. Targeted analysis of

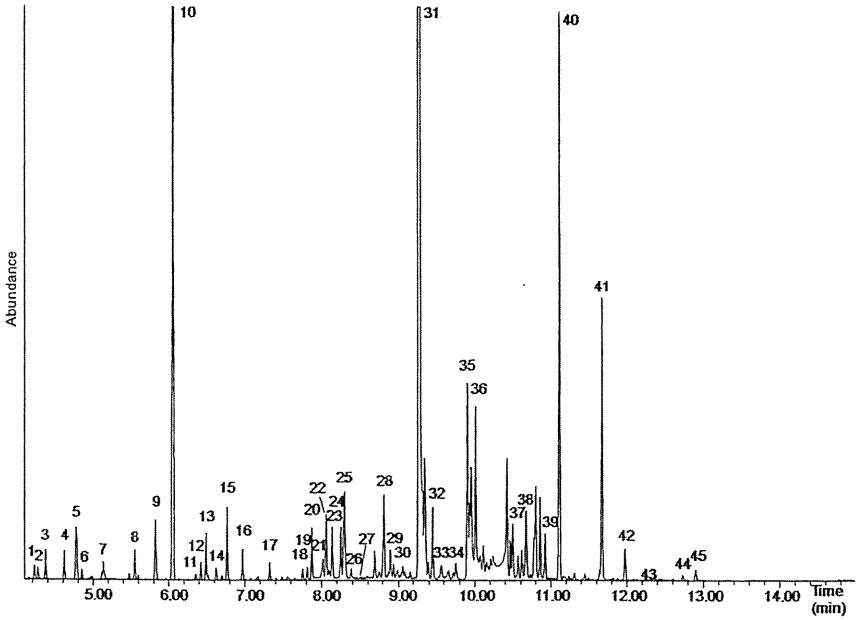


Figure 2. TIC chromatogram of trimethylsilyl derivatives of metabolites from urine (case 2). Major component of each major peak: 1, lactate-2; 2, 2-hydroxyisobutyrate-2; 3, glycolate-2; 4, alanine-2; 5, glycine-2; 6, oxalate-2; 7, sulfate-2; 8, β-aminoisobutyrate-2; 9, urea-2; 10, phosphate-3; 11, phenylacetate-1; 12, succinate-2; 13, 2,2-dimethylsuccinate-2 (IS); 14, N2-uracil-2 (IS); 15, serine-3; 16, threonine-3; 17, 2-deoxytetronate-3; 18, malate-3; 19, threitol-4; 20, erythritol-4; 21, D3-methionine-2 (IS); 22, 5-oxoproline-2; 23, threonate-4; 24, erythronate-4; 25, creatinine-3; 26, 2-hydroxyphenylacetate-2; 27, phenyllactate-2; 28, phenylalanine-2; 29, 4-hydroxyphenylacetate-2; 30, asparagine-3; 31, xylitol-5; 32, 2-hydroxyundecanoate-2 (IS); 33, cis-aconitate-3; 34, glutamine-3; 35, fructose-5; 36, citrate-4; 37, gluconate-4; 38, histidine-3; 39, glucose-5; 40, gluconate-6; 41, urate-4; 42, heptadecanoate-1 (IS); 43, tryptophan-3; 44; D4-cystine-4 (IS); 45, pseudouridine-5.

patients were off TPN, these metabolite levels were normal or significantly lower: in case 1, only a very mild amino aciduria was present; and, in case 2, tyrosine and methionine were increased (Table 1).

The incidence of PKU and PAH heterozygotes is 1/70 000 and 1/130, respectively, in Japan. In case 1, 13 exons of the PAH gene and its flanking intronic regions were screened using denaturing high-performance liquid chromatography (HPLC),[8] and exons 7 and 12 were sequenced as described.[9] No mutations were found except for a V245V silent mutation in exon 7; thus, it was unlikely that this patient was heterozygous for a PAH deficiency. Because of the patient's early death in case 2, we could not perform a mutation analysis for PAH.

BH4 is an essential cofactor for PAH and other enzymes, as shown in Fig. 1. BH4 deficiency causes severe neurological

Table 1. Abnormality *n* of metabolites and Phe concentrations

Abnormality *n* of metabolites in urine									Phe conc.		
Analysis	Phe[a]	2HPA[b]	PL[c]	PA[d]	Tyr[e]	Trp[f]	Met[g]	Gluc[h]	Urine[i]	Plasma[j] (mM)	Serum[k] (mM)
Case 1											
1st	0.9	2.2	−0.7	1.7	0.9	−1.1	−2.7	2.0	14	0.09	
2nd	5.7	10.2	7.0	4.6	1.0	−0.5	6.5	3.3	124	1.10	0.72
Case 2											
1st	7.8	10.5	4.9	5.7	2.4	3.0	2.6	2.3	310	2.67	
2nd	4.8	3.5	3.7	1.6	5.2	3.0	5.1	<10.0	81	0.73	

The levels of 2-hydroxyphenylacetate, phenylacetate, and phenyllactate were obtained relative to the internal standard, 2,2-dimethylsuccinate, and amino acids, by stable isotope dilution. The levels of urinary metabolites in age-matched healthy individuals were $\log_{10}$-transformed, and the mean and SD were obtained. The abnormality *n* in [mean above *n* × SD] is shown for metabolites, [a]phenylalanine, [b]2-hydroxyphenylacetate, [c]phenyllactate, [d]phenylacetate, [e]tryosine, [f]tryptophan, [g]methionine, and [h]glucose, at the 1st and 2nd metabolic profiling. [i]Urine Phe concentrations are expressed as mmol/mol creatinine. [j]Plasma concentration was estimated from that in urine according to Boulos *et al.*[6] [k]Serum concentration was determined in case 1, for which serum was obtained.

DOI: 10.1002/rcm

the GC/MS-based urine metabolome including amino acids and organic acids, obtained in a single run, enables rapid understanding of the metabolic state of the patients under TPN with medication. This highly sensitive and specific GC/MS-based metabolomics is an essential approach for global personalized medicine.

Acknowledgements

The authors wish to acknowledge Dr. N. Takizawa, National Sanatorium Toyama Hospital Japan, for his help in this study.

REFERENCES

1. Matsumoto I, Kuhara T. *Mass Spectrom. Rev.* 1996; **15**: 43.
2. Blau N, Thöny B, Cotton RGH, Hyland K. In *The Metabolic and Molecular Bases of Inherited Diseases*, (8th edn) Scriver CR, Beaudet AL, Sly WS, Valle D (eds). McGraw-Hill: New York, 2001; 1725–1776.
3. Scriver CR, Kaufman S. In, *The Metabolic and Molecular Bases of Inherited Diseases*, (8th edn) Scriver CR, Beaudet AL, Sly WS, Valle D (eds). McGraw-Hill: New York, 2001; 1667–1724.
4. Kuhara T. *J. Chromatogr. B.* 2001; **758**: 3.
5. Kuhara T. *Mass Spectrom. Rev.* 2005; **24**: 814.
6. Boulos M, Boulat O, Van Melle G, Guignard JP, Matthieu J. *Biol. Neonate* 2004; **86**: 6.
7. Costello PM, Beasley MG, Tillotson SL, Smith I. *Eur. J. Pediatr.* 1994; **153**: 260.
8. Okano Y, Asada M, Kang Y, Nishi Y, Hase Y, Oura T, Isshiki G. *Hum. Genet.* 1998; **103**: 613.
9. Brautigam S, Kujat A, Kirst P, Seidel J, Luleyap HU, Froster UG. *Mol. Genet. Metab.* 2003; **78**: 205.
10. Blau N, Bonafe L, Thony B. *Mol. Genet. Metab.* 2001; **74**: 172.
11. Bonafé L, Thöny B, Leimbacher W, Kierat L, Blau N. *Clin. Chem.* 2001; **47**: 477.
12. Evans SJ, Wynne-Williams TC, Russell CA, Fairbrother A. *Lancet* 1986; **ii**: 1404.
13. Shintaku H, Niederwieser A, Leimbacher W, Curtius HC. *Eur. J. Pediatr.* 1988; **147**: 15.
14. Sawabe K, Suetake Y, Nakanishi N, Wakasugi K, Hasegawa H. *Mol. Genet. Metab.* 2005; **86** (Suppl 1): S133.
15. Sawabe K, Suetake Y, Wakasugi K, Hasegawa H. *Mol. Genet. Metab.* 2005; **86** (Suppl 1): S145.
16. Millot F, Dhondt JL, Hayte JM, Bauters F. *Am. J. Pediatr. Hematol. Oncol.* 1992; **14**: 276.
17. Millot F, Dhondt JL, Mazingue F, Mechinaud F, Ingrand P, Guilhot F. *Pediatr. Res.* 1995; **37**: 151.
18. Blau N, Curtius AC, Kierat L, Leupold D, Kohne E. *J. Pediatr.* 1989; **115**: 661.

Movement Disorders
Vol. 24, No. 15, 2009, pp. 2289–2308

Letters to the Editor Related to New Topics

Plasma Phenylalanine Level in Dopa-Responsive Dystonia

DYT5 is a condition characterized by levodopa (L-dopa) responsive dystonia (DRD) that shows childhood onset and marked diurnal fluctuation. Patients with DYT5 have heterozygous mutations in the GCH1 gene, which codes for GTP cyclohydrolase I (GTPCH), a rate-limiting enzyme in tetrahydrobiopterin (BH_4) synthesis.[1] BH_4 is a cofactor for aromatic amino acid hydroxylases including tyrosine hydroxylase (TH), phenylalanine hydroxylase (PAH), and tryptophan hydroxylase.[2] In patients with DYT5, production of dopamine is suppressed due to the decrease of BH_4 because TH is a rate-limiting enzyme in dopamine synthesis. The lack of BH_4 may also affect the activity of PAH, and patients with complete GTPCH deficiency show hyperphenylalaninemia. However, hyperphenylalaninemia has not been reported in patients with DYT5.[3] Therefore, we examined blood phenylalanine levels in patients with DYT5 compared with controls to determine whether the decrease of BH_4 in DYT5 affects PAH activity.

Blood samples for analysis of amino acids were obtained from seven patients with DRD[4] and heterozygous mutations of GCH1, 24 patients with dopa nonresponsive dystonia (non-DRD), and 12 controls. The samples were collected in a tube containing EDTA, and plasma was obtained for analysis of phenylalanine and tyrosine levels using an auto-amino acid analyzer (HS-3000; Hitachi, Tokyo, Japan). All data are expressed as means ± SD. The data were analyzed by analysis of variance (ANOVA) with multiple comparison using the Bonferroni method. A significant difference was defined as a P value < 0.05.

The phenylalanine and tyrosine levels in the plasma of patients with DYT5, patients with non-DRD, and controls are shown in Figure 1. The phenylalanine levels in the DYT5 (81.1 ± 26.6 μmol/L), non-DRD (60.5 ± 14.5 μmol/L), and control (58.7 ± 9.1 μmol/L) groups were all within the normal range. However, the phenylalanine level in patients with DYT5 was significantly higher than those in the other groups ($P = 0.027$ by ANOVA). Multiple comparison also indicated that the level of plasma phenylalanine in patients with DYT5 was significantly higher than those in patients with non-DRD ($P = 0.043$) and in controls ($P = 0.040$). There was no significant difference in the plasma phenylalanine levels between patients with non-DRD and controls. There were no significant differences in plasma tyrosine levels among all the groups.

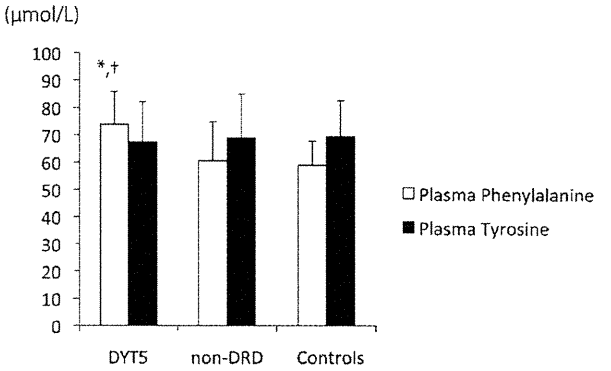


FIG. 1. Plasma phenylalanine and tyrosine levels. The plasma phenylalanine levels (open boxes) and tyrosine levels (closed boxes) are shown for patients with DYT5, patients with non-DRD, and controls. Error bars indicate standard deviations. *$P < 0.05$ (DYT5 vs. non-DRD). †$P < 0.05$ (DYT5 vs. controls).

BH_4 deficiency causes hyperphenylalaninemia and a decrease of dopamine production, because it suppresses the activity of PAH and TH. Patients with a homozygous mutation in the GCH1 gene are reported to show hyperphenylalaninemia, in addition to DRD. Patients with DYT5 having only a heterozygous mutation in GCH1 also show symptoms of DRD, but do not have hyperphenylalaninemia. Our results indicated that blood phenylalanine levels in patients with DYT5 are within the normal range, but are higher than those in controls, which suggests that the activity of PAH is partially affected by the decrease in BH_4 in DYT5.

GTPCH is regulated by BH_4 itself and phenylalanine via GTPCH feedback regulatory protein (GFRP).[5] An increase of phenylalanine induces GFRP to upregulate GTPCH activity, whereas an increase of BH_4 downregulates GTPCH activity via GFRP. Hyland et al. has reported prolonged hyperphenylalaninemia after oral phenylalanine loading in patients with DRD,[6] which suggests that decreased BH_4 production due to mutations in GTPCH restrict the catalysis of excessive phenylalanine by PAH. However, a defect in GFRP or in the interaction between GTPCH and GFRP would cause the same results. Our results indicate that the phenylalanine level in patients with DYT5 differs from those in controls without phenylalanine loading, which suggests that a partial defect of GTPCH affects the activity of PAH.

Author Roles: Hiroki Fujioka, MD, PhD: correcting samples, Performing statistical analysis. Haruo Shintaku, MD: correcting samples, supervising. Satoshi Kudo, PhD: measuring amino acid concentration. Tsunekazu Yamano, MD: supervising.

Potential conflict of interest: Hiroki Fujioka received the Grants-in-Aid for Scientific Research by the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 20790771). Hiroki Fujioka and Haruo Shintaku received Grants-in-Aid for Scientific Research by the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 17591109).

Published online 20 October 2009 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mds.22774

Hiroki Fujioka, MD, PhD*
Haruo Shintaku, MD
Satoshi Kudo, PhD
Tsunekazu Yamano, MD
Department of Pediatrics
Osaka City University Graduate School of Medicine
Osaka, Japan
**E-mail: hfujioka@msic.med.osaka-cu.ac.jp*

Hiroki Fujioka, MD, PhD
Osaka City Fukushima-Ward
Public Health and Welfare Center
Fukushima-Ward Office
Osaka, Japan

References

1. Ichinose H, Ohye T, Takahashi E, et al. Hereditary progressive dystonia with marked diurnal fluctuation caused by mutations in the GTP cyclohydrolase I gene. Nat Genet 1994;8:236–242.
2. Shintaku H. Disorders of tetrahydrobiopterin metabolism and their treatment. Curr Drug Metab 2002;3:123–131.
3. Blau N, Ichinose H, Nagatsu T, et al. A missense mutation in a patient with guanosine triphosphate cyclohydrolase I deficiency missed in the newborn screening program. J Pediatr 1995;126: 401–405.
4. Ohta E, Funayama M, Ichinose H, et al. Novel mutations in the guanosine triphosphate cyclohydrolase 1 gene associated with DYT5 dystonia. Arch Neurol 2006;63:1605–1610.
5. Yoneyama T, Hatakeyama K. Decameric GTP cyclohydrolase I forms complexes with two pentameric GTP cyclohydrolase I feedback regulatory proteins in the presence of phenylalanine or of a combination of tetrahydrobiopterin and GTP. J Biol Chem 1998; 273:20102–20108.
6. Hyland K, Fryburg JS, Wilson WG, et al. Oral phenylalanine loading in dopa-responsive dystonia: a possible diagnostic test. Neurology 1997;48:1290–1297.

Hybrid Cars May Interfere with Implanted Deep Brain Stimulators

Clinicians and patients are always concerned about potential interference between external electromagnetic fields and implantable pacemaker devices. In a recent *New York Times* article, concern was raised about emitted "magnetic fields" from hybrid cars and association with various diseases such as childhood leukemia.[1] We report a case of a patient with deep brain stimulator placement for Parkinson's disease who developed unusual symptoms possibly related to stimulator malfunction while riding in a hybrid car.

A 73-year-old man with history of tremor-dominant Parkinson's disease (PD) underwent bilateral subthalamic nucleus deep brain stimulator (STN DBS) placement. One month later, initial stimulator programming was performed, and he complained of symptoms of severe nausea, dizziness, and palpitations while driving the 4- to 5-hour journey home in a 2008 hybrid Toyota Prius car. The patient's wife had to stop the car multiple times as he felt so ill. Prior to initial programming, the patient was able to drive and ride in the Prius without any problems. After stimulator activation, the patient complained of reproducible symptoms of nausea, dizziness, lightheadedness, and cardiac palpitations when sitting in the front passenger seat. He noticed that the symptoms worsened when both the gasoline engine and electric motor were running or when the car battery was charging. The symptoms spontaneously resolved when he exited the car and never occurred when he was in his truck, which is a nonhybrid vehicle. The symptoms also improved when he manually turned off his stimulator while inside the Prius or when he moved to the back seat. These symptoms did not occur at any other time. On interrogation of his stimulator 4 weeks after initial DBS programming, seven activations were noted with only two that were accounted by the patient turning the pulse generator off and on manually. The internal pulse generators (IPGs), however, had been on 99% of the time.

The patient experienced the worst symptoms when sitting in the front seat of the Prius and when the car battery was being charged, suggesting that the electromagnetic field emitted might be interfering with his neurostimulator settings. There were multiple unaccounted activations on interrogation of the stimulator, although the IPGs were on 99% of the time. He did not get symptoms in a nonhybrid car or in the Prius when his IPG was off. We have observed similar symptoms when the voltage of an STN neurostimulator was increased rapidly. We hypothesize that the device was turning off and on rapidly, with voltage surges, thus causing the patient's symptoms. This is the first documented case of a hybrid vehicle, potentially interfering with the IPG settings in a subject with PD and STN DBS. There has been controversy over the effect of electromagnetic fields generated by hybrid vehicles on cardiac pacemakers and implantable defibrillators. Patients with cardiac pacemakers have complained of similar symptoms as the ones which our patient experienced when inside a hybrid car or near its smart key device (internet message boards).[2] In the Prius owner's manual, Toyota warns that people with implanted pacemakers or cardiac defibrillators should keep away from the vehicle smart entry and start system antennas.[3] Currently, the manual does not comment specifically about DBS. We recommend that such patients should not drive a Prius or other hybrid vehicle until more research and data are available for theirs and others safety. Whether they are safe as passengers remains to be proven. Further investigation should include measurement of all electromagnetic fields and frequencies generated in hybrid and electric cars, including the Toyota Prius, and evaluating for deep brain stimulator device interference or malfunction.

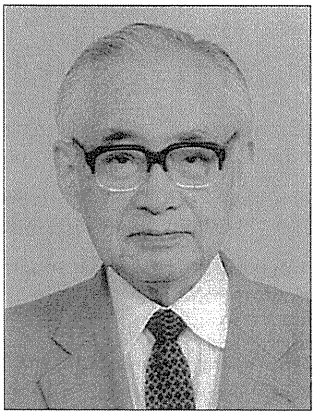
Charlene Chen, MD
Wendy Cole, RN MSN
Department of Neurology and Neurological Sciences
Stanford University
Stanford, California, USA

Potential conflicts of interest: The authors report no conflict of interest.
Published online 18 September 2009 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mds.22739

Autosomal Dominant GTP Cyclohydrolase I (AD GCH 1) Deficiency (Segawa Disease, Dystonia 5; DYT 5)

Masaya Segawa, MD

Autosomal dominant GTP cyclohydrolase I (AD GCH 1) deficiency (Segawa disease) is an autosomal dominant dopa responsive dystonia caused by heterozygous mutation of the GCH 1 gene located on 14q22.1-q22.2. Although a number of mutations have been reported, the change remains highly stable within families, and causes a decrease in the tyrosine hydroxylase protein at the nigrostriatal (NS)-dopamine (DA) neuron terminal. In addition, decreased tetrahydrobiopterin levels early in the development affect DA receptors age-dependently, and produce a spectrum of specific symptoms attributed to neuronal changes traced to processes in the development of the NS-DA neuron, related striatal projection neurons, and the output projection of the basal ganglia. *(Chang Gung Med J 2009;32:1-11)*



Dr. Masaya Segawa

Key words: Segawa disease, postural dystonia, action dystonia, GTP cyclohydrolase I, dopa responsive dystonia

Definition and classification

Autosomal dominant (AD) GTP cyclohydrolase I (GCH 1) deficiency or Segawa disease is a dopa responsive dystonia (DRD) caused by heterozygous mutation of the GCH 1 gene, located on chromosome 14q22.1 to q22.2.[1] This disease was called 'hereditary progressive basal ganglia disease' in case reports of two girls who were cousins.[2] But after experience with an adult case with a 43 year clinical course, this disease was confirmed as dystonia and was published under the name 'hereditary progressive dystonia with marked diurnal fluctuation (HPD)'.[3] Prior to identification of the causative gene of HPD, this disease was called Segawa syndrome,[4] including the recessive type, which was later clarified as recessive tyrosine hydroxylase (TH) deficiency. In the 1990s, it was also called DRD.[5,6] Recently it was classified as DYT 5 with recessive TH deficiency or was called Segawa disease. In this paper, I review the clinical characteristics, laboratory findings and pathophysiology.

Clinical signs and symptoms

Clinical symptoms are characterized by age dependency both in the initial signs and the clinical course.[1] The age of onset is in childhood around 6 years. However, there are patients who have an onset in adulthood[7] or at ages older than fifty years.[1] Childhood onset cases start with postural dystonia of a lower extremity, mostly with talipes equinovarus,

From the Segawa Neurological Clinic for Children, Tokyo, Japan.
Received: Sep. 28, 2007; Accepted: Apr. 29, 2008
Correspondence to: Dr. Masaya Segawa, Segawa Neurological Clinic for Children. 2-8 Surugadai Kanda, Chiyoda-ku, Tokyo 101-0062, Japan. Tel.: 81-3-3294-0371; Fax: 81-3-3294-0290; E-mail: segawa@segawa-clinic.jp

but may start with dystonic posture of one upper extremity at a slightly later age. However, some patients show rigorous dystonic movements; action dystonia, besides postural dystonia. The action dystonia occurs later than postural dystonia from at around 8 years old. It appears commonly as action retrocollis and in some with oculogyric crisis. Writer's cramp or torticollis, that is, focal or segmental dystonia, may appear in adulthood. Postural tremor appears later in the upper extremities mostly after the age of 10 years. Adult onset patients start with hand tremor and gait disturbance due to generalized rigidity. Some start with writer's cramp.

Asymmetry is a characteristic feature and is observed in dystonia, rigid hypertonus and tremor, irrespective of age at onset. Dystonia and tremor show marked diurnal fluctuation, that is, they aggravate towards the evening and recover markedly or nearly completely in the morning after sleep. But these fluctuations are minimal or not apparent in adult onset patients. Patients with onset in early childhood show stagnation of body height with the onset of dystonia and have a short stature of around minus 2 standard deviations by the late teens. This is not observed in patients with onset after adolescence.

Locomotion is preserved and psychomental activities are usually not affected, even in the advanced stages. However some patients show symptoms caused by decreased 5HT activities such as autistic features, depressive state or migraine. Patients with compound heterozygotes show hypotonia, failure in locomotion and delay in mental and motor development.[8] Clinical courses are also characterized by age dependency. Postural dystonia of one lower extremity in childhood expands to all limbs and develops to generalized dystonia by the middle of the teens, and the grade of rigidity aggravates progressively toward the early twenties. However, the progression attenuates from the late teens and after 30 years, the symptoms become stationary. With the attenuation of the progression, the grade of diurnal fluctuation decreases and becomes in the stationary stage. However tremor, with onset in the early teens, expands to all limbs and the trunk with age and this progression is observed until 30 years.

The presence of action dystonia depends on the family. Thus, clinically, Segawa disease is classified into two types, the postural dystonia type, and the action dystonia type, with association of vigorous dystonic movements.[1] Patients with action dystonia type may show focal or segmental dystonia in adulthood. Adult onset patients are usually observed in families with action dystonia. Phenotypical variation, which was reported after discovery of the causative gene,[8-11] depends on symptoms observed in patients with action dystonia.[1] There are families with anticipation in the ages at onset and phenotypes, while others show identical features or marked variation in ages at onset or phenotypes irrelevant of the generation.[1]

Neurological examination

Muscle stretch reflexes demonstrate rigid hypertonus in Segawa disease. But it is not a plastic rigidity, and repeating the test produces fluctuation in the tonus. The tremor is a high frequency postural tremor (8-10 Hz), but a parkinsonian, resting tremor is not observed. Adult onset patients may show resting tremors of lower frequency. However, the tremor of Segawa disease disappears with a stretch reflex, that is, it does not appear as cogwheel rigidity.

These clinical signs show asymmetry, but the pattern of side predominance of the sternocleidomastoideus (SCM) against that of extremity muscles differs between rigidity and tremor. That is, it is contralateral to that of the extremities for rigidity, but ipsilateral for tremor. However, in adult onset cases the side of predominance of the rigid hypertonus is ipsilateral between the SCM and the muscles of the extremities.[12] Bradykinesia or postural instability appears with advancing stages of dystonia. However, as locomotion is preserved, a freezing phenomenon is not observed. The tendon reflexes are brisk and ankle clonus may be observed. However the plantar reflexes are flexor, although some patient exhibit a "striatal toe sign"; a sign associated with dysfunction of the basal ganglia. There are neither cerebellar signs nor sensory disturbances. Psychomental activities are preserved normally.

Investigations

Biochemical studies

Cerebrospinal fluid (CSF) examination reveals low levels of homovanillic acid.[13-17] But characteristic, and pathognomonic features are marked decrease (20-29% of normal levels) of both biopterin and neopterin.[17-20] A moderate reduction of these pteri-

dine metabolites is also observed (about 30-50% of normal levels) in asymptomatic carriers.[21]

The activity of GCH 1 in the mononuclear blood cells of patients is less than 20% of that in healthy individuals, while asymptomatic carriers reach 30 to 40% of normal levels.[22] Phenylalanine loading tests in both child and adult patients reveal a six-hour increase in phenylalanine levels.[23] In addition, the phenylalanine to tyrosine ratios remain elevated during the post-loading period, while biopterin levels decline.[22] However, these tracts tend to show false negative results.

Neuroimaging studies

Magnetic resonance imaging and computed tomography scans of the brain show no abnormalities. Positron emission tomography (PET) scanning also demonstrates normal or low normal uptake levels in both [18F] dopa[24-26] and [11C] raclopride PET[26,27] in symptomatic subjects. [11C] N-spiperone PET revealed a mild increase in receptor binding,[28] but there was no increase in receptor binding in follow-up PET analysis after seven months of levodopa therapy.[29] (1R)-2-Carbβomethoxy-3β-(4-[123I] iodophenyl) tropane ([123I] β-CIT) single photon emission computed tomography (SPECT) scanning was normal.[30]

Neurophysiological studies

Polysomnography (PSG) reveals abnormalities restricted in the phasic motor components of sleep[3,31,32] while sleep structure, percent sleep stage and other parameters modulated by the brainstem aminergic neurons and the cholinergic neurons are preserved normally.[3] These changes include a decrease in the number of gross movements (GMs), and twitch movements (TMs); and abnormality in the pattern of occurrence of GMs against sleep stages. These phasic components of sleep are modulated by the basal ganglia and the nigrostriatal (NS)-dopamine (DA) neurons. The numbers of TMs during rapid eye movement (REM) sleep reflect the activities of the NS-DA neurons.[31,32] Normally, the number of REM-associated TMs decreases with age and shows incremental nocturnal variation with the sleep cycle.[31] In AD GCH 1 deficiency the age-related nocturnal variations of the TMs are preserved, but the number of TMs decreases to approximately 20% or less of normal values.[31]

Abnormalities in the patterns of GMs differ between the postural dystonia and action dystonia types, and that of the latter suggests DA-D_2 receptor supersensitivity.[32] Evaluation of saccadic eye movements reveals abnormalities in both visually guided saccades (VGS) and memory guided saccades (MGS), and implicates involvement of both the direct and indirect pathways.[33,34] Besides exaggeration of the nigro-collicular inhibition associated with slowing in both saccades, disinhibition of the superior colliculi is postulated by failure in suppression of unnecessary saccade in MGS tasks. This suggests hypofunction of the striatal indirect pathway which disfacilitates the output of the basal ganglia. These abnormalities of MGS are more marked in the action dystonia type than the postural dystonia type. However, in adult onset patients only MGS are affected with preservation of the VGS (Segawa unpublished data).

Supracranial magnetic stimulation was normal, showing preservation of the corticospinal tract.[35] Paired pulse transcranial magnetic stimulation showed normal short-interval intra -cortical inhibition of the motor cortex in postural type AD GCH 1 deficiency.[36] This suggests that reduction of GABAergic inhibition of the thalamo-cortical pathway may not contribute to generation of dystonia in postural type AD GCH 1 deficiency.

Brain pathology and histochemistry

An autopsy case reported by Rajput et al[37] was later revealed to be AD GCH 1 deficiency by gene analysis of the brain.[38] Neuropathological examination revealed no demonstrable changes in the substantia nigra (SN) except for a decrease in melanin pigment, particularly in the ventral tier of the pars compacta.[37] Histochemically, DA content is reduced markedly in the striatum but mildly in the pars compacta in the SN.[39] Similar to Parkinson's disease (PD), the reduction is greater in the putamen than in the caudate nucleus, and subregionally, more in the rostral caudate and the caudal putamen.[39] In contrast to PD, AD GCH 1 deficiency shows a greater DA loss in the ventral subdivision of the rostral caudate than its dorsal counterpart, and the activity and protein content of TH are decreased only in the striatum, while they are within the normal range in the SN.[39] There are marked reductions of total biopterin (84%) and neopterin (62%) in the putamen, despite normal

concentrations of aromatic acid-decarboxylase, DA transporter and vesicular monoamine transporter.[40] A post-mortem study on an asymptomatic carrier showed modest reductions of TH protein (52%) and DA (44%), despite marked reduction of striatal biopterin (by 82%).[41]

Molecular biological studies

The causative gene of AD GCH 1 deficiency is the GCH 1 gene located on 14q22.1-q22.2.[22] Although more than one hundred independent mutations have now been identified in the coding region of GCH 1, the locus of mutation differs among families but are identical in one family.[12,22,42] The rate of mutant GCH 1 mRNA production against normal RNA was 28% in one patient but was 8.3% in an asymptomatic carrier.[43,44] Molecular analysis has been unable to determine mutations in the coding region of the gene in approximately 40% of subjects with AD GCH 1 deficiency.[45] In some of these subjects, abnormalities in intron genomic deletion,[42,46] a large gene deletion,[47] an intragenic duplication or inversion of GCH 1, and mutation in an as yet undefined regulatory gene modifying enzyme function are suspected.[45]

Treatment and prognosis

In most cases, a dose of 20 mg/kg per day of plain levodopa without a decarboxylase inhibitor alleviates the symptoms completely.[11,48] Some patients starting treatment with plain levodopa before the age of 10 years tend to have a decreased response after around 13 years.[49] This is due to an increase in decarboxylation in the intestine from around these ages.[49] To these patients levodopa with a decarboxylase inhibitor alleviates the symptoms completely with doses of 4 to 5 mg/kg/day. In a few patients, choreic movements develop with a rapid increase in dosage or with administration of a higher dose of levodopa in the initial stage of treatment.[11] In patients with action dystonia, action retrocollis and oculogyric crisis may be aggregated by initial doses.[32] In patients with the compound heterozygote, aggravation of dystonia with the initial dosage is prominent.[8] In these patients the unfavorable symptoms disappear with a decrease of the dose. After titration to an optimal dosage by starting with a smaller dose and slowly increasing it, levodopa shows sustained and favorable effects without side effects.[8,11] Levodopa is effective without any relation to age of onset and length of the clinical course, and improves short stature if administrated before puberty.[49] However, in cases of action dystonia and adult onset cases, levodopa does not always show complete effects.

Anticholinergic drugs may have a marked and prolonged effect, but do not afford complete relief, either clinically or polysomnographically.[50] It does not improve the tremor.[32,50] Amantadine has proven beneficial for levodopa-related chorea.[51] Tetrahydrobiopterin (BH_4) monotherapy is not favorable[18,52-55] but there are a few patients who show complete remission after administration of BH_4 in addition to levodopa.[52] In one patient with the compound heterozygote, administration of BH_4 was necessary for complete recovery.[8]

Pathophysiological considerations

Why patients with heterozygous gene abnormalities develop symptoms

In the pathogenetic mechanisms of dominant inheritance, a classic dominant negative effect[56,57] and destabilizing effect[58] have been considered. The ratio of mutant/wild-type GCH 1 mRNA in lymphocytes is higher in an affected individual than an unaffected heterozygote, and varies depending on the locus of the mutation.[44,58,59] Furthermore, the ratio differs among affected individuals in some families, depending on the locus of the mutation.[43,58] Thus the degree and the pattern of inactivation of the normal enzyme by the mutant gene differ among the loci of the mutation and may cause inter- and intra-familial variation in the phenotype as well as the rate of penetrance.

Why TH is rather selectively affected

In AD GCH 1 deficiency, TH appears to be preferentially affected when compared to tryptophan hydroxylase. This could be explained by the difference in distribution of GCH 1 mRNA in DA and 5HT neurons,[60] the destabilization of the molecule of TH or impairment of axonal transport.[41] However, a difference in the Km value for TH and tryptophan hydroxylase[61] is most probable. With the heterozygotic mutant gene, BH_4 decreases partially in AD GCH 1 deficiency. Thus TH with a higher affinity to BH_4 is affected rather selectively. In molecular conditions with marked decreases of BH_4, as in

the compound heterozygote, both tryptophan hydroxylase and TH are affected, producing symptoms induced by deficiencies of the 5HT neurons. Or that is, TH has higher affinity to BH4. In Segawa disease with heterozygous mutant gene, the BH4 level is partially decreased, so TH may be affected selectively.

How histochemistry findings relate to clinical symptoms

A complete and sustained response to levodopa suggests a functional lesion restricted to the NS-DA neurons in AD GCH 1 deficiency.[1,48,62] PET studies show that main lesion is decrease of TH activities in the striatum or the terminal of the NS-DA neuron and that, DA-D_2 receptors and DA transporters are not involved in the pathophysiologies of this disease. These are confirmed by neuropathological and histochemical studies.[37,39] Histochemical studies further showed the main lesion with decreases of TH or DA in the ventral area of the caudate nucleus.[39] In the rostral caudate in particular, the medial/ventral portions, the striosomes/patches or D_1 direct pathways are more numerous, whereas in the dorsal/lateral portions, the matrix compartment is more homogenous.[63,64] Thus histochemical findings suggest that the DA loss in AD GCH 1 deficiency is more prominent in the striosomes/patches compartment, the terminal for the D_1 receptor.[39] TH activity of the NS-DA neurons shows age-related decrement and circadian oscillation in the terminals,[65] but these age and state-dependent variations are not observed in the pars compacta of the SN.[66] The age-related clinical course and diurnal fluctuation correlate to the age and circadian variation of the activities of TH in the synaptic terminals of the NS-DA neurons in the caudate.[65] PSG suggested that the TH activities at the terminal follow the decremental age and incremental nocturnal variation of normal individuals with low levels but without progressive decrement of the activities. These features and the results of PET scan confirm that AD GCH 1 deficiency is not a progressive or degenerative disorder and the NS-DA neurons preserve their fundamental functions. These implicate childhood onset, age-related clinical course and diurnal fluctuation.

How particular symptoms of GCH 1 deficiency develop in childhood

Study of GCH 1 activities in stimulated mononuclear blood cells shows age-dependent decrement of the activities in the first three decades of life.[67] Putaminal biopterin levels increase in the postnatal period, reaching a plateau at 1 to 13 years of age, before declining in adulthood.[68] These results imply that pteridine metabolism has a critical period beginning early in infancy and extending to early childhood. This shows the important roles of GCH 1 and BH_4 for neuronal development in the first and the second decades of life.[69] Several processes have been considered in the loss of striatal TH protein with normal preservation in the SN. BH_4 may control protein stability rather than expression.[40] Animal experiments revealed stabilization of TH protein by co-expression of GCH 1[70] and loss of TH protein but not of TH mRNA in the brains of BH_4 deficient mice.[71] Clinically it is suggested that the D_1 direct pathways mature earlier than the D_2 indirect pathways.[72] Dopa-responsive growth arrest seen in children with AD GCH 1 deficiency is a reflection of tuberoinfundibular D_4 receptor involvement. The D_4 receptor belongs to the D_2 receptor family, which, however, matures early among D_2 families.[73] Thus, the DA neuron modulated by pteridine metabolism might regulate DA receptors that mature early in the developmental course.

Phenotypical variation is caused by involvement of different DA neurons and serotonergic neurons

Tremor is levodopa responsive but develops independently from symptoms of postural dystonia. A difference in the side predominance of tremor in the SCM and extremities from that in dystonic hypertonus suggests a different pathophysiology of tremor from that of postural dystonia and implies that the responsible lesion is downstream of the striatum for tremor. As tremor is dopa responsive, involvement of the DA neurons innervating to the subthalamic nucleus (STN) with D_1 receptors[74,75] is postulated. PET findings revealing preservation of function of D_2 receptors[36] support this hypothesis, and suggest that the striatal indirect pathway does not play a role in the generation of symptoms.

This also confirms the unresponsiveness of tremor to anticholinergics. In addition, the response of the tremor to stereotactic thalamotomy targeting the ventrolateral (VL) thalamic nucleus, which was performed in the era before levodopa,[76] suggests involvement of the ascending pathway to the VL

nucleus of the thalamus. Given that the ascending pathways to the thalamus develop later than the descending pathways, increasing age may be a factor for development of tremor.[1,77]

The side of torticollis and the predominant side of SCM in adult onset cases in families with action dystonia are ipsilateral to the predominant side of rigidity. These implicate the involvement of the DA neurons innervating to the STN in these phenotypes. Involvement of the DA neurons innervating to the STN could explain the ipsilateral involvement of the hypertones between STM and extremity nucleus and also PSG findings suggesting D_2 receptor-upward regulation observed in patients with action dystonia.[32] This also postulates the incomplete response to levodopa observed in some cases of action dystonia and adult onset cases because activation of the D_2 receptors of the striatal indirect pathway by levodopa causes suppression of the STN.

The hypotonia and failure in locomotion observed in patients with compound heterozygotes[11] are considered to be a deficiency of 5HT regulated activities.[10] Preservation of interlimb coordination or locomotion in AD GCH 1 deficiency without symptoms of 5HT deficiency may depend on preservation of the descending output of the basal ganglia to the pedunculopontine nucleus.[1,77,78]

Gender differences are still under consideration

Segawa disease has a gender preference for females.[1] In our series with 28 gene-proved patients from 15 families, the ratio was 25:3.[1] Two studies estimating the ratio of symptomatic carriers revealed identical results with penetrance of gene mutations of 87% and 87% for females and 38% and 35% for males, respectively.[1,79] A gender difference in the base levels of GCH 1 in the mononuclear blood cells suggested by Ichinose et al[22] is yet to be confirmed. Thus, the marked female predominance might depend on a genetically determined gender difference in the DA neurons.[80]

Postulates for the pathophysiology of AD GCH 1 deficiency

Partial decrement of BH_4 caused by partial GCH 1 deficiency induces decrement of TH protein in the ventral area of the striatum, resulting in development of particular symptoms through the DA D_1 receptors and the neuronal tracts of the basal ganglia which mature early along with the developmental variation of the enzyme. In childhood, it causes disfacilitation of the D_1 striatal direct pathway, and disinhibits the descending output projection of the internal segment of the globus pallidus and pars reticulata of the SN, which suppress the reticulospinal tract and the superior colliculus. These may cause postural dystonia with exaggeration of the tendon reflexes without extensor plantar reflexes and abnormalities in voluntary saccades. However, with involvement of the DA neuron innervating to the STN with the D_1 receptor, the ascending outputs to the thalamus are disfacilitated and develop tremor and action dystonia later, depending on the functional maturation of the ascending output. As the STN develops early functionally, it causes abnormalities on PSG which mimic D_2 receptor supersensitivity in childhood cases with action dystonia. Because in the state with D2 receptor supersensitivity the STN is suppressed by disinhibition of the external segment of the globus palidus. This situation is the same as disfacilitation of the STN by decreased DA activities innervating to this nucleus. Furthermore, this process also disinhibits the superior colliculus and involves failure in suppression of unnecessary saccade in memory guided tasks. The focal and segmental dystonia observed in patients with action dystonia are caused by dysfunction of the motor cortex due to disinhibition of the thalamo-cortical pathway. Considering the ipsilateral involvement of the predominant side of rigidity in the SCM and extremities, this pathway is postulated to involve in adult onset patients. Preservation of VGS in adult onset cases suggest that the direct pathway and the descending output, which mature at early ages, are not involved in the pathophysiology in these patients. This also relates to absence of postural dystonia in adult onset patients. The pathophysiology of the postural and action dystonia types are shown in Figs 1A and 1B.

Diagnosis

Diagnosis of AD GCH 1 deficiency is usually not difficult in the setting of characteristic clinical symptoms. As gene studies show false negative results, estimation of GCH 1 activity in peripheral mononuclear cells gives a definite diagnosis, but this is technically complicated. Thus, estimation of neopterin and biopterin levels in the CSF is most reliable for diagnosis.

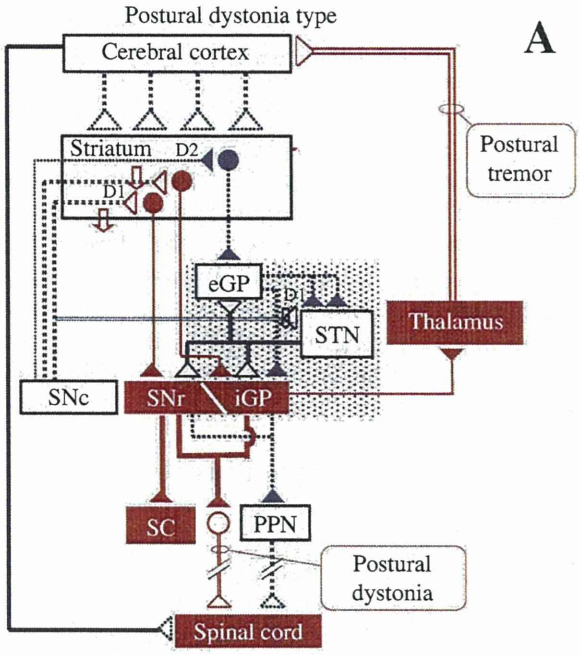


Fig. 1A Postural dystonia type. Abbreviations: eGP: external segment of the globus pallidus; iGP: internal segment of the globus pallidus; STN: subthalamic nucleus; SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; SC: superior colliculus; PPN: pedunculopontine nucleus. The red background shows the structures involved; Symbols: Red solid, single and open lines and triangles: pathways and terminals involved in pathophysiology; The width shows degree of activities; Dotted lines: pathways not involved in pathophysiology; Single line: inhibitory neuron; Open line: excitatory neuron; Closed triangle: inhibitory neuron; Open triangle: excitatory neuron; Shaded region with dots: the area of the circuit for postural tremor.

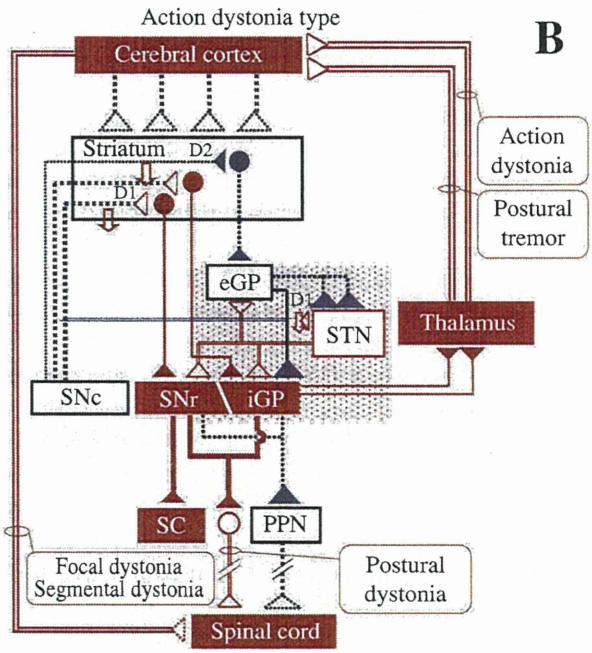


Fig. 1B Action dystonia type. Abbreviations: eGP: external segment of the globus pallidus; iGP: internal segment of the globus pallidus; STN: subthalamic nucleus; SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; SC: superior colliculus; PPN: pedunculopontine nucleus. The red background shows the structures involved. Symbols: Red solid, single and open lines and triangles: pathways and terminals involved in pathophysiology; The width shows degree of activities; Dotted lines: pathways not involved in pathophysiology; Single line: inhibitory neuron; Open line: excitatory neuron; Closed triangle: inhibitory neuron; Open triangle: excitatory neuron; Shaded region with dots: the area of the circuit for postural tremor.

Differential diagnosis

All children with gait disturbance and limb dystonia with asymmetry should be evaluated for AD GCH 1 deficiency. The differential diagnosis includes Wilson's disease, pantothenate kinase-associated neurodegeneration (Hallervorden-Spatz disease), hereditary spastic paraplegia and cerebral palsy. AD GCH 1 deficiency is often misdiagnosed as hereditary spastic paraplegia. The differentiation of AD GCH 1 deficiency from these disorders is usually not difficult with careful clinical examination.

Cases of axial torsion dystonia in childhood, including early onset autosomal dominant torsion dystonia (DYT1), can be differentiated clinically from AD GCH 1 deficiency by evaluating the side predominantly involved between the SCM and muscles of the extremities; that is, it is contralateral in AD GCH 1 deficiency, while it is ipsilateral in dystonias with axial torsion. DYT1 also has two clinical types, postural dystonia and action dystonia.[81] The involved neuronal pathways of the basal ganglia for postural dystonia are the same in both diseases. However, in the action dystonia of DYT1, the striatal indirect pathways are involved with preservation of the STN. This may relate to the presence of motor tricks in DYT1 with action dystonia, while it is not observed in the action dystonia of AD GCH 1 deficiency. However, it should be taken into considera-

tion that adult onset patients in families with the action dystonia type of DYT1, have focal or segmental dystonia, not general dystonia as in adult onset patients in families with the action dystonia type AD GCH 1 deficiency. Differentiation of these patients with a levodopa loading test is recommended, in addition to biochemical and molecular biological studies.

In addition to AD GCH 1 deficiency, dopa-responsive dystonia is also observed in recessive deficiency of pteridine metabolism, recessive TH deficiency (recessive DYT 5) and juvenile parkinsonism (JP).

All of the recessively inherited disorders of pteridine metabolism develop levodopa responsive dystonia caused by a decrease of BH_4 in infancy and early childhood, as in AD GCH 1 deficiency. However, they show levodopa non-responsive postural hypotonia and psychomental disturbances which are caused by deficiency of 5HT activities. Interestingly, recessive sepiapterin reductase deficiency only shows action dystonia[82] suggesting involvement of the DA neurons innervating to the STN. Patients with recessive TH deficiency show ptosis, hyperperspiration and psychomotor disturbances due to noradrenalin (NA) deficiency.[83] Some patients show features almost identical to AD GCH 1 deficiency and the disease responds well to levodopa,[84] however the diurnal fluctuation is not marked as in AD GCH 1 deficiency and patients later develop parkinsonian symptoms due to involvement of D_2 receptors. This is because in recessive TH deficiency, the terminals of DA neurons innervating to the dorsal area of the striatum are involved.[85] For a definite diagnosis of these disorders, estimation of pteridine metabolites and catecholamine metabolites in the CSF is necessary.

JP appears as dystonia when it occurs in childhood to early teens.[86] Although the dystonia of JP responds briskly to levodopa, dyskinesia develops soon after levodopa is started, so it is necessary to start with Dopa agonists in these patients. At this point, JP which is caused by the *parkin* gene (PARK2) is a particularly important disease to differentiate from AD GCH 1 deficiency when it occurs at early ages.

Some patients with AD GCH 1 deficiency develop symptoms later in life (e.g. the fifties and sixties), with tremor, rigidity and gait disturbance but without dystonia. In these patients diurnal fluctuation is not observed and the disease is often misdiagnosed as PD. However, the tremor in these cases is mainly postural and clinical features are milder with minimal progression and without cogwheel rigidity. The most important clinical sign for the differential diagnosis is the side of the predominance of the rigidity between the SCM and the extremities. That is, it is ipsilateral in these patients in contrast to contralateral involvement in JP and PD. Normal preservation of the voluntary saccade in PARK 2 is also diagnostic. However, for definite diagnosis of PARK2, gene analysis is necessary.

REFERENCES

1. Segawa M, Nomura Y, Nishiyama N. Autosomal dominant guanosine triphosphate cyclohydrolase I deficiency (Segawa disease). Ann Neurol 2003;54 Suppl 6:S32-45.
2. Segawa M, Ohmi K, Itoh S, Aoyama M, Hayakawa H. Childhood basal ganglia disease with remarkable response to L-dopa: hereditary basal ganglia disease with marked diurnal fluctuation. Shinryo 1971;24:667-72.
3. Segawa M, Hosaka A, Miyagawa F, Nomura Y, Imai H. Hereditary progressive dystonia with marked diurnal fluctuation. Adv Neurol 1976;14:215-33.
4. Deonna T. DOPA-sensitive progressive dystonia of childhood with fluctuations of symptoms--Segawa's syndrome and possible variants. Results of a collaborative study of the European Federation of Child Neurology Societies (EFCNS). Neuropediatrics 1986;17:81-5.
5. Nygaard TG, Marsden CD, Duvoisin RC. Dopa-responsive dystonia. Adv Neurol 1988;50:377-84.
6. Calne DB. Dopa-responsive dystonia. Ann Neurol 1994;35:381-2.
7. Nomura Y, Segawa M. Intrafamilial and interfamilial variations of symptoms of Japanese hereditary progressive dystonia with marked diurnal fluctuation. In: Segawa M, ed. Hereditary Progressive Dystonia with Marked Diurnal Fluctuation. Carnforth, U.K.: The Parthenon Publishing Group, 1993:73-96.
8. Furukawa Y, Kish SJ, Bebin EM, Jacobson RD, Fryburg JS, Wilson WG, Shimadzu M, Hyland K, Trugman JM. Dystonia with motor delay in compound heterozygotes for GTP-cyclohydrolase I gene mutations. Ann Neurol 1998;44:10-6.
9. Bandmann O, Nygaard TG, Surtees R, Marsden CD, Wood NW, Harding AE. Dopa-responsive dystonia in British patients: new mutations of the GTP-cyclohydrolase I gene and evidence for genetic heterogeneity. Hum Mol Genet 1996;5:403-6.
10. Segawa M, Hoshino K, Hachimori K, Nishiyama N, Nomura Y. A single gene for dystonia involves both or

either of the two striatal pathways. In: Nicholson LFB, Faull RLM, eds. The Basal Ganglia VII. New York: Kluwer Academic / Plenum Publishers, 2002:155-63.
11. Segawa M, Nomura Y, Kase M. Diurnally fluctuating hereditary progressive dystonia. In: Vinken PJ, Bruyn GW, Klawans HL, eds. Handbook of Clinical Neurology, Vol. 5 (49): Extrapyramidal Disorders. Amsterdam: Elsevier Science Publishers B. V., 1986:529-39.
12. Segawa M. Hereditary progressive dystonia with marked diurnal fluctuation. Brain Dev 2000;22 Suppl 1:S65-80.
13. Kumamoto I, Nomoto M, Yoshidome H, Osame M, Igata A. 5 cases of dystonia with marked diurnal fluctuation and special reference to homovanillic acid in CSF. Rinsho Shinkeigaku 1984;24:697-702.
14. Maekawa N, Hashimoto T, Sasaki M, Oishi T, Tsuji S. A study on catecholamine metabolites in CSF in a patient with progressive dystonia with marked diurnal fluctuation. Rinsho Shinkeigaku 1988;28:1206-8.
15. Ouvrier RA. Progressive dystonia with marked diurnal fluctuation. Ann Neurol 1978;4:412-7.
16. Shimoyamada Y, Yoshikawa A, Kashii H, Kihira S, Koike M. Hereditary progressive dystonia--an observation of the catecholamine metabolism during L-DOPA therapy in a 9-year-old girl. No To Hattatsu 1986;18:505-9.
17. Fink JK, Barton N, Cohen W, Lovenberg W, Burns RS, Hallett M. Dystonia with marked diurnal variation associated with biopterin deficiency. Neurology 1988;38:707-11.
18. LeWitt PA, Miller LP, Levine RA, Lovenberg W, Newman RP, Papavasiliou A, Rayes A, Eldridge R, Burns RS. Tetrahydrobiopterin in dystonia: identification of abnormal metabolism and therapeutic trials. Neurology 1986;36:760-4.
19. Fujita S, Shintaku H. Etiology and pteridine metabolism abnormality of hereditary progressive dystonia with marked diurnal fluctuation (HPD: Segawa disease). Med J Kushiro City Hosp 1990;2:64-7.
20. Furukawa Y, Nishi K, Kondo T, Mizuno Y, Narabayashi H. CSF biopterin levels and clinical features of patients with juvenile parkinsonism. Adv Neurol 1993;60:562-7.
21. Takahashi H, Snow B, Nygaard T, Yokochi M, Calne D. Fluorodopa PET scans of juvenile parkinsonism with prominent dystonia in relation to dopa-responsive dystonia. In: Segawa M, Nomura Y, eds. Age-related Dopamine-Dependent Disorders. Monographs in Neural Sciences. Vol. 14. Basel: Karger, 1995:87-94.
22. Ichinose H, Ohye T, Takahashi E, Seki N, Hori T, Segawa M, Nomura Y, Endo K, Tanaka H, Tsuji S, Fujita K, Nagatsu T. Hereditary progressive dystonia with marked diurnal fluctuation caused by mutations in the GTP cyclohydrolase I gene. Nat Genet 1997;8:236-42.
23. Hyland K, Fryburg JS, Wilson WG, Bebin EM, Arnold LA, Gunasekera RS, Jacobson RD, Rost-Ruffner E, Trugman JM. Oral phenylalanine loading in dopa-responsive dystonia: a possible diagnostic test. Neurology 1997;48:1290-7.
24. Snow BJ, Okada A, Martin WRW, Duvoisin RC, Calne DB. Positron-emission tomography scanning in dopa-responsive dystonia, parkinsonism-dystonia, and young-onset parkinsonism. In: Segawa M, ed. Hereditary Progressive Dystonia with Marked Diurnal Fluctuation. Carnforth, U.K.: The Parthenon Publishing Group, 1993:181-6.
25. Sawle GV, Leenders KL, Brooks DJ, Harwood G, Lees AJ, Frackowiak RS, Marsden CD. Dopa-responsive dystonia: [18F]dopa positron emission tomography. Ann Neurol 1991;30:24-30.
26. Turjanski N, Weeks R, Sawle GV, Brooks DJ. PET studies of the dopaminergic and opioid function in dopa-responsive dystonic syndromes and Tourette's syndrome. In: Segawa M, Nomura Y, eds. Age-related Dopamine-Dependent Disorders. Monographs in Neural Sciences. Vol. 14. Basel: Karger, 1995:77-86.
27. Leenders KL, Antonini A, Meinck H-M, Weindl A. Striatal dopamine D2 receptors binding in dopa-responsive dystonia and Parkinson's disease. In: Segawa M, Nomura Y, eds. Age-related Dopamine-Dependent Disorders. Monographs in Neural Sciences. Vol. 14. Basel: Karger, 1995:95-100.
28. Kunig G, Leenders KL, Antonini A, Vontobel P, Weindl A, Meinck HM. D2 receptor binding in dopa-responsive dystonia. Ann Neurol 1998;44:758-62.
29. Kishore A, Nygaard TG, de la Fuente-Fernandez R, Naini AB, Schulzer M, Mak E, Ruth TJ, Calne DB, Snow BJ, Stoessl AJ. Striatal D2 receptors in symptomatic and asymptomatic carriers of dopa-responsive dystonia measured with [11C]-raclopride and positron-emission tomography. Neurology 1998;50:028-32.
30. Jeon BS, Jeong JM, Park SS, Kim JM, Chang YS, Song HC, Kim KM, Yoon KY, Lee MC, Lee SB. Dopamine transporter density measured by [123I]beta-CIT single-photon emission computed tomography is normal in dopa-responsive dystonia. Ann Neurol 1998;43:792-800.
31. Segawa M, Nomura Y, Hikosaka O, Soda M, Usui S, Kase M. Roles of the basal ganglia and related structures in symptoms of dystonia. In: Carpenter MB, Jayaraman A, eds. The Basal Ganglia II: Structure and Function - Current Concepts (Advances in Behavioral Biology, Vol 32). New York: Plenum Press, 1987:489-504.
32. Segawa M, Nomura Y, Tanaka S, Hakamada S, Nagata E, Soda M, Kase M. Hereditary progressive dystonia with marked diurnal fluctuation--consideration on its pathophysiology based on the characteristics of clinical and polysomnographical findings. Adv Neurol 1988;50:367-76.
33. Hikosaka O, Sakamoto M, Usui S. Functional properties of monkey caudate neurons. I. Activities related to saccadic eye movements. J Neurophysiol 1989;61:780-98.
34. Hikosaka O, Fukuda H, Kato K, Uetake K, Nomura Y, Segawa M. Deficits in saccadic eye movements in hereditary progressive dystonia with marked diurnal fluctuation. In: Segawa M, ed. Hereditary Progressive Dystonia with

Marked Diurnal Fluctuation. Carnforth, U.K.: The Parthenon Publishing Group, 1993:159-77.
35. Muller K, Homberg V, Lenard HG. Motor control in childhood onset dopa-responsive dystonia (Segawa syndrome). Neuropediatrics 1989;20:185-91.
36. Hanajima R, Nomura Y, Segawa M, Ugawa Y. Intracortical inhibition of the motor cortex in Segawa disease (DYT5). Neurology 2007;68:1039-44.
37. Rajput AH, Gibb WR, Zhong XH, Shannak KS, Kish S, Chang LG, Hornykiewicz O. Dopa-responsive dystonia: pathological and biochemical observations in a case. Ann Neurol 1994;35:396-402.
38. Furukawa Y, Shimadzu M, Rajput AH, Shimizu Y, Tagawa T, Mori H, Yokochi M, Narabayashi H, Hornykiewicz O, Mizuno Y, Kish SJ. GTP-cyclohydrolase I gene mutations in hereditary progressive amd dopa-responsive dystonia. Ann Neurol 1996;39:609-17. Erratum in: Ann Neurol 1996;40:134.
39. Hornykiewicz O. Striatal dopamine in dopa-responsive dystonia: comparison with idiopathic Parkinson's disease and other dopamine-dependent disorders. In: Segawa M, Nomura Y, eds. Age-related Dopamine-Dependent Disorders. Monographs in Neural Sciences. Vol. 14. Basel: Karger, 1995:101-8.
40. Furukawa Y, Nygaard TG, Gutlich M, Rajput AH, Pifl C, DiStefano L, Chang LJ, Price K, Shimadzu M, Hornykiewicz O, Haycock JW, Kish SJ. Striatal biopterin and tyrosine hydroxylase protein reduction in dopa-responsive dystonia. Neurology 1999;53:1032-41.
41. Furukawa Y, Kapatos G, Haycock JW, Worsley J, Wong H, Kish SJ, Nygaard TG. Brain biopterin and tyrosine hydroxylase in asymptomatic dopa-responsive dystonia. Ann Neurol 2002;51:637-41.
42. Nishiyama N, Yukishita S, Hagiwara H, Kakimoto S, Nomura Y, Segawa M. Gene mutation in hereditary progressive dystonia with marked diurnal fluctuation (HPD), strictly defined dopa-responsive dystonia. Brain Dev 2000;22 Suppl 1:S102-6.
43. Hirano M, Tamaru Y, Nagai Y, Ito H, Imai T, Ueno S. Exon skipping caused by a base substitution at a splice site in the GTP cyclohydrolase I gene in a Japanese family with hereditary progressive dystonia dopa responsive dystonia. Biochem Biophys Res Commun 1995;213:645-51.
44. Hirano M, Tamaru Y, Ito H, Matsumoto S, Imai T, Ueno S. Mutant GTP cyclohydrolase I mRNA levels contribute to dopa-responsive dystonia onset. Ann Neurol 1996;40:796-8.
45. Furukawa Y, Kish SJ. Dopa-responsive dystonia: recent advances and remaining issues to be addressed. Mov Disord 1999;14:709-15.
46. Ichinose H, Ohye T, Segawa M, Nomura Y, Endo K, Tanaka H, Tsuji S, Fujita K, Nagatsu T. GTP cyclohydrolase I gene in hereditary progressive dystonia with marked diurnal fluctuation. Neurosci Lett 1995;196:5-8.
47. Furukawa Y, Guttman M, Sparagana SP, Trugman JM, Hyland K, Wyatt P, Lang AE, Rouleau GA, Shimadzu M, Kish SJ. Dopa-responsive dystonia due to a large deletion in the GTP cyclohydrolase I gene. Ann Neurol 2000;47:517-20.
48. Segawa M, Nomura Y. Hereditary progressive dystonia with marked diurnal fluctuation and Dopa-responsive dystonia: Pathognomonic clinical features. In: Segawa M, Nomura Y, eds. Age-related Dopamine-Dependent Disorders. Monographs in Neural Sciences. Vol. 14. Basel: Karger, 1995:10-24.
49. Segawa M, Nomura Y, Yamashita S, Kase M, Nishiyama N, Yukishita S, Ohta H, Nagata K, Hosaka A. Long-term effects of L-dopa on hereditary progressive dystonia with marked diurnal fluctuation. In: Berardelli A, Benecke R, Manfredi M, Marsden CD, eds. Motor Disturbances II. London: Academic Press, 1990:305-18.
50. Kase M, Nomura Y, Igawa C, Ogiso M, Segawa M. A female case of hereditary progressive dystonia with marked diurnal fluctuation with favorable response to anticholinergic drugs for 25 years. Clin Neurol (Tokyo) 1984;22:723.
51. Furukawa Y, Filiano JJ, Kish SJ. Amantadine for levodopa-induced choreic dyskinesia in compound heterozygotes for GCH1 mutations. Mov Disord 2004;19:1256-8.
52. Ibi T, Sahashi K, Watanabe K, Morishima T, Mitsuma T, Fujishiro K, Takahashi A, Hagiwara M, Nagatsu T. Progressive dystonia with marked diurnal fluctuations and tetrahydrobiopterin therapy. Neurol Therap 1991;8:71-5.
53. Ishida A, Takada G, Kobayashi Y, Higashi O, Toyoshima I, Takai K. Serotonergic disturbance in hereditary progressive dystonia--clinical effects of tetrahydrobiopterin and 5-hydroxytryptophan. No To Hattatsu 1988;20:195-9.
54. LeWitt PA, Miller LP, Newman RP, Lovenberg W, Eldridge R, Chase TN. Pterdine cofactor in dystonia: Pathogenic and therapeutic considerations. Neurology 1983;33:161.
55. LeWitt PA, Newman RP, Miller LP, Lovenberg W, Eldridge R. Treatment of dystonia with tetrahydrobiopterin. N Engl J Med 1983;308:157-8.
56. Hirano M, Yanagihara T, Ueno S. Dominant negative effect of GTP cyclohydrolase I mutations in dopa-responsive hereditary progressive dystonia. Ann Neurol 1998;44:365-71.
57. Hwu WL, Chiou YW, Lai SY, Lee YM. Dopa-responsive dystonia is induced by a dominant-negative mechanism. Ann Neurol 2000;48:609-13.
58. Suzuki T, Ohye T, Inagaki H, Nagatsu T, Ichinose H. Characterization of wild-type and mutants of recombinant human GTP cyclohydrolase I: relationship to etiology of dopa-responsive dystonia. J Neurochem 1999;73:2510-6.
59. Ueno S, Hirano M. Missense mutants inactivate guanosine triphosphate cyclohydrolase I in hereditary progressive dystonia. Brain Dev 2000;22 Suppl 1:S111-4.
60. Shimoji M, Hirayama K, Hyland K, Kapatos G. GTP cyclohydrolase I gene expression in the brains of male and female hph-1 mice. J Neurochem 1999;72:757-64.

61. Davis MD, Ribeiro P, Tipper J, Kaufman S. "7-tetrahydrobiopterin," a naturally occurring analogue of tetrahydrobiopterin, is a cofactor for and a potential inhibitor of the aromatic amino acid hydroxylases. Proc Natl Acad Sci USA 1992;89:10109-13.
62. Segawa M. Hereditary progressive dystonia (HPD) with marked diurnal fluctuation. Adv Neurol Sci 1981;25:73-81.
63. Graybiel AM, Ragsdale CW Jr. Histochemically distinct compartments in the striatum of human, monkeys, and cat demonstrated by acetylthiocholinesterase staining. Proc Natl Acad Sci USA 1978;75:5723-6.
64. Gibb WRG. Selective pathology, disease pathogenesis and function in the basal ganglia. In: Kimura J, Shibasaki H, eds. Recent Advances in Clinical Neurophysiology. Amsterdam: Elsevier Science B.V., 1996:1009-15.
65. McGeer EG, McGeer PL. Some characteristics of brain tyrosine hydroxylase. In: Mandel J, ed. New Concepts in Neurotransmitter Regulation. New York, London: Plenum Press, 1973:53-68.
66. Steinfels GF, Heym J, Strecker RE, Jacobs BL. Behavioral correlates of dopaminergic unit activity in freely moving cats. Brain Res 1983;258:217-28.
67. Hibiya M, Ichinose H, Ozaki N, Fujita K, Nishimoto T, Yoshikawa T, Asano Y, Nagatsu T. Normal values and age-dependent changes in GTP cyclohydrolase I activity in stimulated mononuclear blood cells measured by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 2000;740:35-42.
68. Furukawa Y, Kish SJ. Influence of development and aging on brain biopterin: implications for dopa-responsive dystonia onset. Neurology 1998;51:632-4.
69. Shintaku H. Early diagnosis of 6-pyruvoyl-tetrahydropterin synthase deficiency. Pteridines 1994;5:18-27.
70. Leff SE, Rendahl KG, Spratt SK, Kang UJ, Mandel RJ. In vivo L-DOPA production by genetically modified primary rat fibroblast or 9L gliosarcoma cell grafts via coexpression of GTPcyclohydrolase I with tyrosine hydroxylase. Exp Neurol 1998;151:249-64.
71. Sumi-Ichinose C, Urano F, Kuroda R, Ohye T, Kojima M, Tazawa M, Shiraishi H, Hagino Y, Nagatsu T, Nomura T, Ichinose H. Catecholamines and serotonin are differently regulated by tetrahydrobiopterin. A study from 6-pyruvoyltetrahydropterin synthase knockout mice. J Biol Chem 2001;276:41150-60. Epub 2001 Aug 21.
72. Segawa M. Development of the nigrostriatal dopamine neuron and the pathways in the basal ganglia. Brain Dev 2000;22 Suppl 1:S1-4.
73. Nair VD, Mishra RK. Ontogenic development of dopamine D4 receptor in rat brain. Brain Res Dev Brain Res 1995;90:180-3.
74. Kreiss DS, Anderson LA, Walters JR. Apomorphine and dopamine D(1) receptor agonists increase the firing rates of subthalamic nucleus neurons. Neuroscience 1996;72:863-76.
75. Kreiss DS, Mastropietro CW, Rawji SS, Walters JR. The response of subthalamic nucleus neurons to dopamine receptor stimulation in a rodent model of Parkinson's disease. J Neurosci 1997;17:6807-19.
76. Segawa M, Nomura Y, Takita K, Narabayashi H. Pallidotomy and thalamotomy on a case with hereditary progressive dystonia with marked diurnal fluctuation. Mov Disord 1998;13 Suppl 2:S165.
77. Segawa M, Nomura Y. Rapid eye movements during stage REM are modulated by nigrostriatal dopamine neurons? In: Bernardi G, Carpenter MB, Chiara GD, Morelli M, Stanzione P, eds. Advances in Behavioral Biology, vol. 39, The Basal Ganglia III. New York: Plenum Press, 1991:663-71.
78. Segawa M. Progress in Segawa's Disease. In: Mizuno Y, Fisher A, Hanin I, eds. Mapping the Progress of Alzheimer's and Parkinson's Disease. New York: Kluwer Academic / Plenum Publishers, 2002:353-9.
79. Furukawa Y, Lang AE, Trugman JM, Bird TD, Hunter A, Sadeh M, Tagawa T, St George-Hyslop PH, Guttman M, Morris LW, Hornykiewicz O, Shimadzu M, Kish SJ. Gender-related penetrance and de novo GTP-cyclohydrolase I gene mutations in dopa-responsive dystonia. Neurology 1998;50:1015-20.
80. Reisert I, Pilgrim C. Sexual differentiation of monoaminergic neurons--genetic or epigenetic? Trends Neurosci 1991;14:468-73.
81. Nomura Y, Ikeuchi T, Tsuji S, Segawa M. Two phenotypes and anticipation observed in Japanese cases with early onset torsion dystonia (DYT1) - pathophysiological consideration. Brain Dev 2000;22 Suppl 1:S92-101.
82. Neville BG, Parascandalo R, Farrugia R, Felice A. Sepiapterin reductase deficiency: a congenital dopa-responsive motor and cognitive disorder. Brain 2005; 128 Pt 10:2291-6. Epub 2005 Jul 27.
83. Hoffmann GF, Assmann B, Brautigam C, Dionisi-Vici C, Haussler M, de Klerk JB, Naumann M, Steenbergen-Spanjers GC, Strassburg HM, Wevers RA. Tyrosine hydroxylase deficiency causes progressive encephalopathy and dopa-nonresponsive dystonia. Ann Neurol 2003;54 Suppl 6:S56-65.
84. Furukawa Y, Graf WD, Wong H, Shimadzu M, Kish SJ. Dopa-responsive dystonia simulating spastic paraplegia due to tyrosine hydroxylase (TH) gene mutations. Neurology 2001;56:260-3.
85. Kondo T, Mori H, Sugita Y, Mizuno Y, Yokochi M. Juvenile parkinsonism - A clinical, neuropathological and biochemical study -. Mov Disord 1997;12 Suppl 1:32.
86. Yamamura Y, Hamaguchi Y, Uchida M, Fujioka H, Watanabe S. Parkinsonism of early-onset with diurnal fluctuation. In: Segawa M, ed. Hereditary Progressive Dystonia with Marked Diurnal Fluctuation. Carnforth, U.K.: The Parthenon Publishing Group, 1993:51-9.

RESEARCH ARTICLE

AMERICAN JOURNAL OF
medical genetics

Various Types of *LRP5* Mutations in Four Patients With Osteoporosis-Pseudoglioma Syndrome: Identification of a 7.2-kb Microdeletion Using Oligonucleotide Tiling Microarray

Satoshi Narumi,[1] Chikahiko Numakura,[2] Takashi Shiihara,[2,3] Chizuru Seiwa,[4] Yasuyuki Nozaki,[5] Takanori Yamagata,[5] Mariko Y. Momoi,[5] Yoriko Watanabe,[6] Makoto Yoshino,[6] Toyojiro Matsuishi,[6] Eriko Nishi,[7] Hiroshi Kawame,[7] Tsutomu Akahane,[8] Gen Nishimura,[9] Mitsuru Emi,[10] and Tomonobu Hasegawa[1]*

[1]Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan

[2]Department of Pediatrics, Yamagata University School of Medicine, Yamagata, Japan

[3]Division of Neurology, Gunma Children's Medical Center, Shibukawa, Japan

[4]Department of Pediatrics, Yamagata Prefectural Comprehensive Rehabilitation and Education Center, Yamagata, Japan

[5]Department of Pediatrics, Jichi Medical University, Tochigi, Japan

[6]Department of Pediatrics, Kurume Graduate School of Medicine, Kurume, Japan

[7]Division of Medical Genetics, Nagano Children's Hospital, Nagano, Japan

[8]Department of Orthopedic Surgery, Nagano National Hospital, Nagano, Japan

[9]Department of Radiology, Tokyo Metropolitan Kiyose Children's Hospital, Tokyo, Japan

[10]DNA Chip Research, Inc., Yokohama, Japan

Received 28 May 2009; Accepted 2 October 2009

Osteoporosis-pseudoglioma syndrome (OPS; OMIM 259770) is an autosomal-recessive genetic disorder characterized by severe osteoporosis and visual disturbance from childhood. Biallelic mutations in the low-density lipoprotein receptor-related protein 5 gene (*LRP5*) have been frequently detected, while a subset of patients had only one or no detectable mutation. We report on the clinical and molecular findings of four unrelated Japanese patients with the syndrome. The four patients had typical skeletal and ocular phenotypes of OPS, namely severe juvenile osteoporosis and early-onset visual disturbance, with or without mental retardation. We undertook standard PCR-based sequencing for *LRP5* and found four missense mutations (p.L145F, p.T244M, p.P382L, and p.T552M), one nonsense mutation (p.R1534X), and one splice site mutation (c.1584+1G>A) among four OPS patients. Although three patients had two heterozygous mutations, one had only one heterozygous splice site mutation. In this patient, RT-PCR from lymphocytic RNA demonstrated splice error resulting in 63-bp insertion between exons 7 and 8. Furthermore, the patient was found to have only mutated RT-PCR fragment, implying that a seemingly normal allele did not express LRP5 mRNA. We then conducted custom-designed oligonucleotide tiling microarray analyses targeted to a 600-kb genome region harboring *LRP5* and discovered a 7.2-kb microdeletion encompassing exons 22 and 23 of *LRP5*. We found various types of *LRP5* mutations, including an exon-level

How to Cite this Article:
Narumi S, Numakura C, Shiihara T, Seiwa C, Nozaki Y, Yamagata T, Momoi MY, Watanabe Y, Yoshino M, Matsuishi T, Nishi E, Kawame H, Akahane T, Nishimura G, Emi M, Hasegawa T. 2010. Various types of *LRP5* mutations in four patients with osteoporosis-pseudoglioma syndrome: Identification of a 7.2-kb microdeletion using oligonucleotide tiling microarray.
Am J Med Genet Part A 152A:133–140.

*Correspondence to:
Tomonobu Hasegawa, M.D., Ph.D., Department of Pediatrics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail: thaseg@sc.itc.keio.ac.jp
Published online 22 December 2009 in Wiley InterScience (www.interscience.wiley.com)
DOI 10.1002/ajmg.a.33177

deletion that is undetectable by standard PCR-based mutation screening. Oligonucleotide tiling microarray seems to be a powerful tool in identifying cryptic structural mutations.

Key words: osteoporosis-pseudoglioma syndrome; low-density lipoprotein receptor-related protein 5 (LRP5); mutation; microdeletion; comparative genomic hybridization

INTRODUCTION

Osteoporosis, a leading cause of fractures, is a growing public health burden associated with the aging of the world's population. Osteoporosis is characterized by low bone mineral density (BMD) and bone microarchitectural alterations. Because BMD is highly heritable trait [heritability estimates, 0.6–0.8, Cummings and Melton, 2002], elucidating the genetic regulation of BMD has a crucial role in unveiling molecular mechanisms of osteoporosis.

There are a handful of genetic forms of osteoporosis showing Mendelian inheritance, which usually onset earlier and cause more profound bone fragility. These syndromes are rare but have provided valuable insights into bone homeostasis. Osteoporosis-pseudoglioma syndrome (OPS; OMIM 259770) is an example of such a syndromic osteoporosis. OPS is usually associated with biallelic inactivating mutations in the low-density lipoprotein receptor-related protein 5 gene [*LRP5*; Gong et al., 2001], of which gene product acts as a coreceptor for transducing signals by Wnt proteins [Pinson et al., 2000; Tamai et al., 2000; Wehrli et al., 2000]. Clinically, OPS is hallmarked by severe osteoporosis and visual disturbance from childhood [Frontali et al., 1985]. Osteoporosis typically onsets in juvenile period and results in long-bone fractures, vertebral compression fractures, kyphoscoliosis, deformity of extremities, and short stature. Congenital or early-onset visual disturbance arises from the degeneration of vitreoretinal tissue and manifests a wide range of ophthalmologic problems including phthisis bulbi, retinal detachment, falciform folds, and microphthalmia. Of interest, inactivating *LRP5* mutations is also associated with a genetic ophthalmologic disorder named familial exudative vitreoretinopathy (FEVR; MIM 601813) [Jiao et al., 2004; Toomes et al., 2004]. Some FEVR patients were confirmed to have low BMD [Qin et al., 2005]. It is also noteworthy that several single-nucleotide polymorphisms in *LRP5* locus are associated with variation of BMD among general population [Koay et al., 2004; Mizuguchi et al., 2004; Urano et al., 2004; Koller et al., 2005; van Meurs et al., 2006; Richards et al., 2008].

While evidence for the importance of *LRP5* in bone homeostasis is growing, understanding of molecular genetics of OPS remains still incomplete. In the most intensive clinical/genetic study that enrolled 37 OPS patients, 26 patients were shown to have biallelic *LRP5* mutations but the remaining 11 patients were shown to have only one (n = 4) or no mutation (n = 7) [Ai et al., 2005]. Thus, roughly 30% of OPS patients cannot be explained by the current genetic model. Here, we report on the clinical and molecular findings of four unrelated Japanese patients with OPS. Standard PCR-based sequencing revealed that three patients had biallelic heterozygous *LRP5* mutations, but one had only one heterozygous mutation. In the course of RNA studies, we unexpectedly found that a seemingly normal allele of the patient did not express LRP5 mRNA. We then conducted custom-designed oligonucleotide tiling microarray analyses targeted to a 600-kb genome region harboring *LRP5* and identified a novel 7.2-kb microdeletion encompassing exons 22 and 23 of *LRP5*. We exemplify the usefulness of oligonucleotide tiling microarray in detecting cryptic structural mutations that are undetectable by standard PCR-based mutation screening.

PATIENT REPORTS

Approval for this study was obtained from the institutional review board of Keio University School of Medicine. We obtained written informed consent for molecular studies from the patients and/or parents. The four probands are unrelated and of Japanese origin. No consanguinity was reported among the parents. Clinical information is summarized in Table I.

Patient 1, an 18-year-old boy, was born at term after uneventful pregnancy and delivery. Reportedly, he could not follow objects well with eyes in his early infancy. Ophthalmologic examination revealed microphthalmia, cataract, and retinal detachment in both eyes. His vision had been limited throughout the follow-up period. He had moderate mental retardation: intelligence quotient (IQ) score on the Wechsler Intelligence Scale for Children was 45 at age 11 years. Between ages 7 and 13 years, he had three fractures in the distal diaphysis of the right femur caused by low-impact trauma. Skeletal radiographs showed severe osteopenia, platyspondyly in all vertebrae, and slender and osteopenic but not deformed long bones. The BMD of the lumber spine was 0.47 g/cm^2 [−6.3 SD; age- and sex-matched reference by Nishiyama et al., 1999] at age 14 years.

Patient 2, a 23-year-old male, was born at term after uneventful pregnancy and delivery. At age 1 month, his parents noticed leukocoria in his left eye. Ophthalmologic examination revealed microphthalmia and retinal detachment in the left eye and persistent hyperplasia of the primary vitreous in the right eye. His visual acuity was 0.06 right and hand motion left at last visit. He had no mental retardation. Between ages 3 and 11 years, he had a history of eight fractures at the wrist, ankle, and femurs caused by low-impact trauma. At age 9 years, he had compression fractures in his spines (Th7-10, L1) with no recognizable cause. Osteopenia, kyphoscoliosis, and platyspondyly in all vertebrae were shown by radiographs. At age 11 years, he became wheelchair dependent due to multiple compression fractures. He had short stature with adult height of 151.6 cm (−3.2 SD). The BMD of the lumber spine was 0.47 g/cm^2 (−5.6 SD) at age 13 years.

Patient 3, a 10-year-old male, was born at term after uneventful pregnancy and delivery. In his neonatal period, bilateral nystagmus was noted. At age 4 months, ophthalmologic examination revealed severe retinal detachment accompanied by vascular remnants in the vitreous body in the right eye. In the left eye, insufficient development of peripheral retinal vessel, abnormal vascular growth, and optic disc traction were observed. Although he had visual responses in his infancy, abnormal vascular growth in his left eye deteriorated, and he was completely blind at age 3 years. He had moderate mental retardation: his verbal IQ score on the Wechsler Intelligence Scale for Children was 47 at age 7 years. At age 9 years, he reported falling

TABLE I. Clinical Phenotypes of *LRP5* Mutation Carriers

			OPS phenotype				
			Skeletal				
Subjects	Age (years)	*LRP5* genotype	Height (SDS)	Long-bone fractures (N)	BMD (SDS)	Eye	Other remarks
Patient 1	18	p.[T244M] + [P382L]	−1.9	3	**−6.3**[a]	Cataract, retinal detachment, microphthalmia	Mental retardation
Father	49	p.P382L (heterozygous)	−0.5	0	NA	None	
Mother	48	p.T244M (heterozygous)	−1.1	0	NA	None[b]	
Patient 2	23	p.[L145F(+)T552M]	**−3.2**	8	**−5.6**	PHPV, retinal detachment, microphthalmia	
Mother	45	p.L145F (heterozygous)	−1.3	0	**−2.2**	None[b]	
Patient 3	10	p.[P382L] + [R1534X]	−0.7	1	**−11.2**	PHPV, retinal detachment	Mental retardation, muscular hypotonia, joint laxity
Father	50	p.P382L (heterozygous)	+0.2	0	NA	None	
Mother	44	p.R1534X (heterozygous)	−1.3	0	NA	None	
Patient 4	7	p.[E528_V529ins21] + g.[Ex22_Ex23del]	**−2.3**	5	**−7.5**[c]	PHPV, retinal detachment, microphthalmia	Mental retardation
Father	37	g.Ex22_Ex23del (heterozygous)	−0.7	4[d]	**−3.2**	None	
Mother	36	p.E528_V529ins21 (heterozygous)	+0.4	0	−1.7	None	Unilateral glaucoma
Sister	10	p.E528_V529ins21 (heterozygous)	−0.6	0	**−3.4**	None	Ebstein anomaly of heart

BMD, bone mineral density; NA, not available; OPS, osteoporosis-pseudoglioma syndrome; PHPV, persistent hyperplasia of primary vitreous.
Abnormal values are highlighted in bold.
[a]Measured after 2 years of oral bisphosphonate therapy. Data before therapy are unavailable.
[b]Routine fundoscopic examination was normal in these subjects.
[c]Measured after three courses of intravenous bisphosphonate therapy. Sex- and age-matched reference data are unavailable for pretreatment age.
[d]All fractures were caused by moderate-to-severe-impact trauma (e.g., fall down during skiing).

down while skiing resulting in a sacral fracture. No other fractures have been reported. Physical examination at age 10 years showed joint laxity, muscular hypotonia, and increased magnitude of patellar tendon reflex. Skeletal radiographs revealed osteopenia and platyspondyly in all vertebrae. Long bones were not deformed. The lumber spine BMD was 0.17 g/cm^2 (−11.2 SD) at age 10 years.

Patient 4, a 7-year-old boy, was born at 36 weeks of gestation with minor breathing problems. At age 1 month, his parents noticed bilateral leukocorea. Subsequent examination revealed retinal detachment and tumor formation in both eyes, and he was diagnosed as having bilateral persistent hyperplasia of the primary vitreous. His vision deteriorated progressively to complete blindness. He had moderate mental retardation: Japanese Developmental Scale for visually disabled children was 38 at age 3 years. Between ages 3 and 6 years, he had five peripheral fractures (at his femur, wrist, and ankle) and two rib fractures caused by low-impact trauma. Skeletal radiographs at age 5 years showed marked osteopenia and platyspondyly in all vertebrae. The thorax was narrow with slender posterior ribs. Long bones were slender and deformed mildly. He had short stature (height: 100.0 cm at age 5 years; −2.3 SD). The lumber spine BMD was 0.19 g/cm^2 that seemed extremely low (no age-matched reference for the Japanese is available).

MATERIALS AND METHODS

PCR-Based Mutation Screening

We extracted genomic DNA from peripheral blood of the four probands (and family members of each proband if available) by a standard technique. We analyzed 23 coding exons and flanking introns of *LRP5* by PCR and direct sequencing. Primer sequences and PCR conditions are available on request. The sequence was determined by a BigDye Dideoxy Sequence Kit (Applied Biosystems, Foster City, CA) and an automated sequencer ABI3130*xl* (Applied Biosystems). Detected mutations were tested in 100 Japanese control individuals.

RT-PCR

We isolated total RNA from peripheral lymphocytes of Patient 4 and his parents using a PAXgene Blood RNA Kit (Qiagen, Hilden, Germany). Lymphocytic cDNA was synthesized by reverse transcriptase reaction with oligo-dT primer (SuperScript III; Invitrogen, Carlsbad, CA). Fragments spanning exons 6–8 was PCR amplified and sequenced using following primers: 5′-ATT GCC ATC GAC TAC GAC CCG C-3′ and 5′-GCA GCT GGT CAA TGA TGA CGT CC-3′.

Microarray Analyses

DNA samples from Patient 4, the father of Patient 4, and two control individuals were subject to high-density oligonucleotide-based array comparative genomic hybridization (aCGH) assay. We fabricated a custom-designed microarray targeted to a 600-kb genome region harboring *LRP5* at 11q13.2 (Chr11: 67,600,000–68,200,000 [NCBI Build 36.1, hg18]) according to the methods previously described [Barrett et al., 2004; Perry et al., 2008]. In brief, we used Agilent website (http://earray.chem.agilent.com/earray/) to select and design our custom two-chip CGH array; the array contained 4,751 probes of 60-mer in size (Agilent Technologies, Santa Clara, CA), with median spacing 127 bp.

aCGH experiments were performed according to the manufacturer's instructions [de Smith et al., 2007]. In brief, test and reference (NA19000, a Japanese male from HapMap project) genomic DNAs (350 ng per sample) were fluorescently labeled with Cy5 (test) and Cy3 (reference) with a ULS Labeling Kit (Agilent Technologies). For each sample, respective labeling reactions were mixed and then separated prior to hybridizing to each of the two arrays. Labeled test and reference DNAs were combined, denatured, preannealed with Cot-1 DNA (Invitrogen) and blocking reagent (Agilent Technologies), and then hybridized to the arrays for 40 hr in a rotating oven (Agilent Technologies) at 65°C and 20 rpm. After hybridization and washes, the arrays were scanned at 5 μm resolution with an Agilent G2505B scanner. Images were analyzed with Feature Extraction Software 9.5.3.1 (Agilent Technologies), with the CGH-v4-95-Feb07 protocol for background subtraction and normalization.

Determination of the Deletion Breakpoints

A PCR primer pair whose forward primer (5′-CAC CCT TTC TCT CCA CCT GTC TAA T-3′) and reverse primer (5′-GGT CTT CCA TCC CTT CTT TTA GTG A-3′) located in intron 21 and 1.5-kb downstream of exon 23, respectively, was used to amplify and sequence a 0.5-kb genomic fragment containing the breakpoints of the deletion involving exons 22 and 23.

RESULTS

Mutation Screening

Using standard PCR-based sequencing, we detected three novel *LRP5* mutations (c.1145C>T, p.P382L; c.1584+1G>A [splice donor site in intron 7]; and c.1655C>T, p.T552M) and three previously described mutations [c.433C>T, p.L145F, Qin et al., 2005; c.731C>T, p.T244M, Ai et al., 2005; and c.4600C>T, p.R1534X, Gong et al., 2001] in the four OPS patients (Fig. 1A). These mutations were not found in 100 control individuals. The residues affected by missense mutations (Leu145, Thr244, Pro382, and Thr552) were conserved throughout vertebrate evolution (Fig. 1B). Three patients had two heterozygous mutations: p.[T244M] + [P382L] for Patient 1, p.[L145F(+)T552M] for Patient 2, and p.[P382L] + [R1534X] for Patient 3 (Fig. 2). Patient 4 had only one heterozygous splice site mutation (c.1584+1G>A) that was transmitted from the mother.

Genetic analyses for family members revealed seven subjects having a monoalleic mutation (Fig. 2). Clinical phenotypes of those subjects are summarized in Table I.

RT-PCR

To test the effect of c.1584+1G>A on splicing of exon 7, we performed RT-PCR spanning exons 6–8 from lymphocytic cDNA of Patient 4 and the mother. Gel electrophoresis of the PCR products showed a larger fragment than expected in the two subjects (Fig. 3A). Moreover, we unexpectedly found that Patient