Table 2 Comparison of iFGF23 levels and the severity of XLH between the same-sex siblings from each three kindred

	sex	age (years)	iFGF23 (pg/mL)	bone deformity	pretreatment		$TmPO_4/GFR$		therapeutic dosage of PO_4 before 15 y.o. (mg/day)
					IP (mg/dL)	ALP (IU/L)	infantile	childhood	
F1-1	M	12	359.5	NP	1.9	1957	1.88	1.5	150
-2	M	10	286.4	P	2.1	2040	3.18	1.66	240
F2-1	F	3	70.1	P	2.4	1762		1.45	240
-2	F	2	181.6	NP	1.9	2081	2.95		360
F3-1	F	24	84.5	P	1.7	695		1.64	160
-2	F	21	47.5	NP	1.9	813		1.71	140

Two patients from each family (F1~3) are siblings. M: male, F: female P : presented, NP : not presented, y.o.: years old

in all patients except one (47.5 pg/mL in one female who was 21 years old, and >71.2 pg/mL in the other 6 patients). iFGF23 levels in the patients without treatment or whose compliance was poor were as follows: 1) before initiation of therapy with phosphate in four patients, 40.0, 57.8, 83.8, and 71.2 pg/mL, 2) noncompliance for more than six years in two patients, 84.5, 47.5 pg/mL, 3) poor compliance in two; 84.3, 117.1 pg/mL. iFGF23 levels were not significantly different in three patients with and without treatment (83.8 and 71.1, 84.5 and 117.7, 47.5 and 83.6 pg/mL with and without the treatment, respectively).

No correlation between severity of XLH and iFGF23 levels

There was no correlation between iFGF23 levels and any index of severity of XLH, which included gender, age of onset, existence or nonexistence of bone deformity, $TmPO_4/GFR$, serum IP levels, serum ALP levels, therapeutic dose of phosphate (mg/kg/day), and phosphate dosage which increased serum IP levels by 1.5 mg/dL after oral administration (mg/kg/dose). iFGF23 levels were not significantly different between male and female patients (Fig. 3). Even in a kindred in whom the members had the same genetic background, the iFGF23 level in a female member (mother) was higher than those of male members (sons) (Fig. 3). iFGF23 levels did not correlate with age of onset, existence or nonexistence of bone deformity, $TmPO_4/GFR$, serum IP levels, or therapeutic dose of phosphate, even among the same gender siblings in the same kindred (Table 2).

Discussion

The reference range (5^{th} and 95^{th} percentiles) for iFGF23 level for ages 18 years and younger was from 12.9 to 51.2 pg/mL. It was not significantly different from that for adults (11.5 to 48.9 pg/mL). iFGF23 level in healthy children was reported to be higher than that in adults [22]. The difference from our data may be due to small samples and wide variation of iFGF23 levels, considering wide SD range in the previous report as our data.

iFGF23 levels should be determined in serum obtained before oral phosphate administration and a meal. FGF23 may be not only a chronic but also an acute regulator of phosphorus homeostasis. An upward trend was shown in iFGF23 levels after oral phosphate administration in patients with abnormalities in *PHEX*. A change in iFGF23 levels was observed after a meal in two of seven healthy individuals, however no general tendency was observed. It was reported that low and high phosphate diet for more than 4 days reduced and increased FGF23 levels, respectively in both human [18-20] and mouse [21]. This is the first report to determine that a single oral phosphate load also did increase iFGF23 levels within two hours. A reason why no increase was observed in iFGF23 levels after a meal in five healthy individuals may be because blood glucose lowers serum IP levels, or non-standardized diets contained variable amount of phosphate, although changes in serum IP levels were not available.

An iFGF23 level over 40 pg/mL with hypophosphatemia is one of the clinical indicators to help establish a diagnosis of XLH as well as family history, bone deformity, increased ALP, and low $TmPO_4/GFR$. In our study where participants included only patients with XLH who were diagnosed genetically, iFGF23 levels were above the upper limit of the reference range in 19 of 21 patients with abnormalities in *PHEX*. Our human data is congruent to the increased FGF23 levels found in the Hyp mouse. However, in the other two

patients, iFGF23 levels were below the upper limit of the reference range (40.0 and 47.5 pg/mL). The lowest iFGF23 level in patient with XLH was reported to be 38.0 pg/mL [15]. Patients with XLH may be unusual in that their iFGF23 levels were measurable despite hypophosphatemia, considering that highest iFGF23 levels in hypophosphatemic patients without XLH was 35.8 pg/mL in our clinic (data not shown) and reported to be 23.9 pg/mL [15]. Although treating XLH with phosphate and calcitriol was reported to associated with increases in FGF23 concentrations [23], iFGGF23 levels in patients who do not undergo therapy were even higher than in healthy individuals in this study. Although FGF23-related hypophosphatemic disease other than XLH could not be excluded, majority of FGF23-related hypophosphatemic disease are XLH and the treatment for patients with FGF23-related hypophosphatemic disease are identical regardless of the cause.

The reason why iFGF23 levels in human patients with XLH were not consistently high, unlike in the Hyp mouse, remains unknown. Overlaps in FGF23 levels between patients with XLH and healthy control were reported previously, using the c-terminal ELISA assay [24, 25]and the intact FGF23 assay [15, 25, 26]. There are three possible reasons for the overlaps: 1) phosphatonins other than FGF23 such as MEPE, secreted frizzled-related protein-4 (sFRP-4) and FGF7 may be a modifier in the pathogenesis of XLH in patients with abnormalities in *PHEX*, 2) iFGF23 may decrease due to degradation when samples are stored long-term, 3) patients with low iFGF23 levels may have milder symptoms. One patient whose iFGF23 level was 40.0 pg/mL never had treatment, because her symptoms were mild. However, some patients whose iFGF23 levels were below 100 pg/mL were severely affected, and there was no correlation between iFGF23 levels and any of the indicators for the severity of XLH.

Considering that FGF23 is regarded as a major phosphatonin, FGF23 levels are predicted to correlate with the severity of XLH, which was not true in this study. It is reported that serum phosphate levels are negatively correlated with circulating FGF23 levels (C-terminal ELISA assay) in 11 patients with XLH, of which four of the 11 patients were diagnosed solely by clinical means [24]. However, serum phosphate levels did not correlate with iFGF23 levels in this study. Because XLH is an X-linked disorder, iFGF23 levels are expected to be higher in male patients than in female patients. However, even within the same kindred, iFGF23 levels in female patient were higher than in male patients, as was reported previously[25].

There are two possible reasons why iFGF23 levels do not correlate with disease severity in XLH. One is that phosphatonins other than FGF23 may be involved in determining the severity in XLH, as is discussed above. The other is that iFGF23 levels may be affected by treatment with oral phosphate administration. iFGF23 levels were reported to be elevated after long term phosphate loading [15, 18 -21]. However, even when patients undergoing treatment with phosphate are excluded, there was still no correlation between iFGF23 levels and disease severity (data not shown). Furthermore, iFGF23 levels were not significantly different in three patients that were either treated or untreated (83.8 and 71.1, 84.5 and 117.7, 47.5 and 83.6 pg/mL treated and untreated for each patient, respectively) (Fig. 3).

Conclusion

Increasing tendency after single phosphate administration and no general tendency after ad lib diet in iFGF23 were observed. The reference range for children (12.9~51.2 pg/mL) was similar to that for adults. iFGF23 levels were above the reference range in 19 of 21 patients with abnormalities in *PHEX* (>40.0 pg/mL). Relatively high iFGF23 level despite hypophosphatemia is one of the clinical indicators to help establish a diagnosis of XLH when there is also a positive family history of XLH, bone deformity, increased ALP, and a low $TmPO_4/GFR$. iFGF23 did not correlate with any index of severity of XLH.

Acknowledgement

We are grateful to Dr. Dean T. Yamaguchi (associate chief of staff, Research and Development, United States Department of Veterans Affairs Va Greater Los Angeles Healthcare System) for editing the manuscript. We are also grateful to Dr. Seiji Fukumoto (Division of Nephrology & Endocrinology, Department of Medicine, University of Tokyo Hospital), Dr. Hiroshi Hartaya and Dr. Tomoyuki Sakai (Division of Nephrology, Tokyo Metropolitan Children's Medical Center) for provision of serum samples of healthy adults and children for measurement of FGF23 levels.

References

1. ADHR Consortium (2000) Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. *Nat Genet* 26(3):345-348.
2. Feng, J. Q.; Ward, L. M.; Liu, S.; Lu, Y.; Xie, Y.; Yuan, B.; Yu, X.; Rauch, F.; Davis, S. I.; Zhang, S.; Rios, H.; Drezner, M. K.; Quarles, L. D.; Bonewald, L. F.; White, K. E (2006) Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nature Genet* 38: 1310-1315.
3. Lorenz-Depiereux, B.; Bastepe, M.; Benet-Pages, A.; Amyere, M.; Wagenstaller, J.; Muller-Barth, U.; Badenhoop, K.; Kaiser, S. M.; Rittmaster, R. S.; Shlossberg, A. H.; Olivares, J. L.; Loris, C.; Ramos, F. J.; Glorieux, F.; Vikkula, M.; Juppner, H.; Strom, T. M. (2006) DMP1 mutations in autosomal recessive hypophosphatemia implicate a bone matrix protein in the regulation of phosphate homeostasis. *Nature Genet* 38: 1248-1250.
4. Lorenz-Depiereux B, Schnabel D, Tiosano D, Häusler G, Strom TM (2010) Loss-of-function ENPP1 mutations cause both generalized arterial calcification of infancy and autosomal-recessive hypophosphatemic rickets. *Am J Hum Genet* 86(2): 267-272.
5. Levy-Litan V, Hershkovitz E, Avizov L, Leventhal N, Bercovich D, Chalifa-Caspi V, Manor E, Buriakovsky S, Hadad Y, Goding J, Parvari R (2010) Autosomal-recessive hypophosphatemic rickets is associated with an inactivation mutation in the ENPP1 gene. *Am J Hum Genet* 86(2): 273-278.
6. PHEX Locus database: www.phexdb.mcgill.ca
7. Sitara D, Razzaque MS, Hesse M, Yoganathan S, Taguchi T, Erben RG, Jüppner H, Lanske B (2004) Homozygous ablation of fibroblast growth factor-23 results in hyperphosphatemia and impaired skeletogenesis, and reverses hypophosphatemia in Phex-deficient mice. *Matrix Biol* 23(7): 421-432.
8. Rowe PS, de Zoysa PA, Dong R, Wang HR, White KE, Econs MJ, Oudet CL (2000) MEPE, a new gene expressed in bone marrow and tumors causing osteomalacia. *Genomics* 67(1): 54-68.
9. Berndt T, Craig TA, Bowe AE, Vassiliadis J, Reczek D, Finnegan R, Jan De Beur SM, Schiavi SC, Kumar R (2003) Secreted frizzled-related protein 4 is a potent tumor-derived phosphaturic agent. *J Clin Invest* 112(5): 785-794.
10. White KE, Larsson TE, Econs MJ (2006) The roles of specific genes implicated as circulating factors involved in normal and disordered phosphate homeostasis: frizzled related protein-4, matrix extracellular phosphoglycoprotein, and fibroblast growth factor 23. *Endocr Rev* 27(3): 221-241.
11. Shaikh A, Berndt T, Kumar R (2008) Regulation of phosphate homeostasis by the phosphatonins and other novel mediators. *Pediatr Nephrol* 23(8): 1203-2310.
12. Larsson T, Marsell R, Schipani E, Ohlsson C, Ljunggren O, Tenenhouse HS, Jüppner H, Jonsson KB (2004) Transgenic mice expressing fibroblast growth factor 23 under the control of the alpha1(I) collagen promoter exhibit growth retardation, osteomalacia, and disturbed phosphate homeostasis. *Endocrinology* 145(7): 3087-3094.
13. Shimada T, Hasegawa H, Yamazaki Y, Muto T, Hino R, Takeuchi Y, Fujita T, Nakahara K, Fukumoto S, Yamashita T (2004) FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. *J Bone Miner Res* 19(3): 429-435.
14. Yamazaki Y, Okazaki R, Shibata M, Hasegawa Y, Satoh K, Tajima T, Takeuchi Y, Fujita T, Nakahara K, Yamashita T, Fukumoto S (2002) Increased circulatory level of biologically active full-length FGF-23 in patients with hypophosphatemic rickets/osteomalacia. *J Clin Endocrinol Metab* 87(11): 4957-4960.
15. Endo I, Fukumoto S, Ozono K, Namba N, Tanaka H, Inoue D, Minagawa M, Sugimoto T, Yamauchi M, Michigami T, Matsumoto T (2008) Clinical usefulness of measurement of fibroblast growth factor 23 (FGF23) in hypophosphatemic patients: proposal of diagnostic criteria using FGF23 measurement. *Bone* 42(6): 1235-1239.
16. Jonsson KB, ZahradnikR, Larsson T, White KE, Sugimoto T, Imanishi Y, Yamamoto T, Hampson G, Koshiyama H, Ljunggren O, Oba K, Yang IM, Miyauchi A, Econs MJ, Lavigne J, Juppner H (2003) Fibroblast growth factor 23 in oncogenic osteomalacia and X-linked hypophosphatemia. *N Engl J Med* 348: 1656-1663.
17. Razzaque MS (2009) The FGF23-Klotho axis: endocrine regulation of phosphate homeostasis. *Nat Rev Endocrinol* 5(11): 611-619.
18. Ferrari SL, Bonjour JP, Rizzoli R (2005) Fibroblast growth factor-23 relationship to dietary phosphate and renal phosphate handling in healthy young men. *J Clin Endocrinol Metab* 90(3): 1519-1524.
19. Antoniucci DM, Yamashita T, Portale AA (2006) Dietary phosphorus regulates serum fibroblast growth factor-23 concentrations in healthy men. *J Clin Endocrinol Metab* 91(8): 3144-3149.
20. Burnett SM, Gunawardene SC, Bringhurst FR, Jüppner H, Lee H, Finkelstein JS (2006) Regulation of C-terminal and intact FGF-23 by dietary phosphate in men and women. *J Bone Miner Res* 21(8): 1187-1196.
21. Perward F, Azam N, Zhang MYH, Yamashita T, Tenenhouse HS, Portale AA (2005). Dietary and serum phosphorus regulate fibroblast growth factor 23 expression and 1,25-dihydroxyvitamin D metabolism in mice. *Endocrinology* 146: 5358-5364.

22. Yamazaki Y, Imura A, Urakawa I, Shimada T, Murakami J, Aono Y, Hasegawa H, Yamashita T, Nakatani K, Saito Y, Okamoto N, Kurumatani N, Namba N, Kitaoka T, Ozono K, Sakai T, Hataya H, Ichikawa S, Imel EA, Econs MJ, Nabeshima Y (2010) Establishment of sandwich ELISA for soluble alpha-Klotho measurement: Age-dependent change of soluble alpha-Klotho levels in healthy subjects. *Biochem Biophys Res Commun* 398(3): 513-518.
23. Imel EA, DiMeglio LA, Hui SL, Carpenter TO, Econs MJ (2010) Treatment of X-linked hypophosphatemia with calcitriol and phosphate increases circulating fibroblast growth factor 23 concentrations. *J Clin Endocrinol Metab* 95(4): 1846-1850.
24. Weber TJ, Liu S, Indridason OS, Quarles LD (2003) Serum FGF23 levels in normal and disordered phosphorus homeostasis. *J Bone Miner Res* 18(7): 1227-1234.
25. Ito N, Fukumoto S, Takeuchi Y, Yasuda T, Hasegawa Y, Takemoto F, Tajima T, Dobashi K, Yamazaki Y, Yamashita T, Fujita T (2005). Comparison of two assays for fibroblast growth factor (FGF)-23. *J Bone Miner Metab* 23(6): 435-440.
26. Yamazaki Y, Okazaki R, Shibata M, Hasegawa Y, Satoh K, Tajima T, Takeuchi Y, Fujita T, Nakahara K, Yamashita T, Fukumoto S (2002) Increased circulatory level of biologically active full-lenghth FGF-23 in patients with hypophosphatemic rickets/osteomalacia. *J Clin Endocrinol Metab* 87: 4957-4960.

Nonclassic TSH Resistance: *TSHR* Mutation Carriers with Discrepantly High Thyroidal Iodine Uptake

Satoshi Narumi,* Keisuke Nagasaki,* Tomohiro Ishii, Koji Muroya, Yumi Asakura, Masanori Adachi, and Tomonobu Hasegawa

Department of Pediatrics (S.N., T.I., T.H.), Keio University School of Medicine, Tokyo 160-8582, Japan; Division of Pediatrics (K.N.), Department of Homeostatic Regulation and Development, Niigata University Graduate School of Medicine and Dental Sciences, Niigata 951-8510, Japan; and Department of Endocrinology and Metabolism (K.M., Y.A., M.A.), Kanagawa Children's Medical Center, Yokohama 232-8555, Japan

Context: Inactivating mutations in the TSH receptor gene (*TSHR*) cause TSH resistance. Most patients with TSH resistance have low to normal thyroidal radioiodine uptake (RAIU), which is consistent with the physiological knowledge that TSH stimulates iodine uptake. To date, only one *TSHR* mutation-carrying family with discrepantly high RAIU has been reported.

Objective: We aimed to test whether *TSHR* mutation carriers with high RAIU are observed in a cohort of Japanese patients with congenital hypothyroidism (CH).

Subjects and Methods: Twenty-four Japanese CH patients with high RAIU were screened for *TSHR* mutations. The capacities of mutant TSHR to activate Gs- and Gq-coupled signaling pathways were evaluated *in vitro*.

Results: Two patients were found to have biallelic *TSHR* mutations: p.[T145I]+[R450H] in one and p.[R450H]+[I661fs] in the other. The two subjects had permanent CH with slightly high RAIU (41.8 and 43.0%, reference 8–40) but did not have goiter. One had a slightly high perchlorate discharge rate (10%, reference <10). Expression experiments revealed that T145I-TSHR retained partial ability to transduce both Gs- and Gq-coupled pathways, whereas I661fs-TSHR could transduce neither of them. R450H-TSHR had partial ability to transduce Gs-coupled signaling but had abrogated ability to transduce Gq-coupled signaling, indicating that coupling to Gq was dominantly affected.

Conclusions: We show that 8% of Japanese CH patients with high RAIU (two in 24) has inactivating *TSHR* mutations. Expression of this apparently discrepant phenotype, which we term nonclassic TSH resistance, is presumably associated with the characteristic signaling property of the mutant TSHR, namely the Gq-dominant coupling defect. (***J Clin Endocrinol Metab* 96: E1340–E1345, 2011**)

The two major forms of congenital hypothyroidism (CH) are thyroid dysgenesis (TD) and thyroid dyshormonogenesis. These two are discriminated by the following: 1) thyroid morphology: abnormal position and/or size in TD; goiter in dyshormonogenesis, and 2) iodine handling in the thyroid: low radioiodine uptake (RAIU) in TD; high RAIU with or without abnormal iodine discharge after perchlorate administration in dyshormonogenesis.

Nonetheless, a subset of patients can be classified into neither TD nor dyshormonogenesis categories. One example is TSH resistance (1), which is typically caused by inactivating mutations in the TSH receptor gene (*TSHR*) (2). To date, more than 60 biallelic *TSHR* mutation carriers have been reported (3). Their phenotypes fall into two groups seemingly depending on the residual receptor functions (4): nonfunctional alleles (*e.g.* truncating mutations) cause uncompensated TSH resistance, characterized by

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2011-0070 Received January 10, 2011. Accepted May 23, 2011.
First Published Online June 15, 2011

* S.N. and K.N. contributed equally to this work.

Abbreviations: CH, Congenital hypothyroidism; CRE, cAMP-response element; HA, hemagglutinin; HEK, human embryonic kidney; ICL1, first intracellular loop; NFATRE, nuclear factor AT response element; RAIU, radioiodine uptake; TD, thyroid dysgenesis; Tg, thyroglobulin; TSHR, TSH receptor; WT, wild type.

overt hypothyroidism, thyroid hypoplasia, and low RAIU (5), whereas hypomorphic alleles (*e.g.* most missense mutations) produce compensated TSH resistance presenting subclinical hypothyroidism, normal-sized thyroid, and normal RAIU (2). In the clinical setting, the former is recognized as TD, whereas the latter is regarded as idiopathic CH. These clinical/molecular observations have defined the classic phenotypic spectrum of TSH resistance.

Although a vast majority of mutation carriers show the classic phenotypic features, a single mutation-carrying family presenting a dyshormonogenesis-like phenotype has been described: Grasberger *et al.* (6) reported consanguineous siblings with high RAIU who were homozygous for the p.L653V mutation. However, it has been unclear whether this unusual phenotype is restricted to this family or can be seen among a cohort of CH patients. In the present study, we screened *TSHR* mutations in 24 Japanese CH patients with high RAIU.

Subjects and Methods

Subjects

We enrolled subjects with following criteria: 1) having primary CH with a positive newborn screening test result; and 2) having greater than 40% of ^{123}I uptake evaluated at 24 h after radioiodine administration. ^{123}I uptake was evaluated at age 3 yr or older with transient discontinuation of levothyroxine replacement. Thyroid size was expressed as SD from mean height-appropriate thyroid size (7).

We obtained written informed consent for participating in the study from the subjects and/or their parents. The Institutional Review Board of Keio University School of Medicine approved the study.

Mutation detection

Sequencing of *TSHR* was performed as previously described (8). If a patient had a mutation, we further sequenced *TG, TPO, SLC26A4, DUOX2, DUOXA2,* and *IYD*. The primer sequences and PCR conditions are available upon request.

Functional characterization of mutant TSHR

The details of the experimental procedures are described in Supplemental Data, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org.

Expression vectors [untagged or hemagglutinin (HA)-tagged] encoding wild-type (WT)-TSHR were created, and the three mutations (T145I, R450H, and I661fs) were introduced by site-directed mutagenesis. Cells [human embryonic kidney (HEK) 293 or HeLa] were maintained and were subject to DNA transfection as previously described (9).

Activation of G protein-coupled signaling by TSHR (WT or mutant) was studied with use of luciferase assays: cAMP-response element (CRE) (pGL4.29; Promega, Madison, WI) and nuclear factor AT response element (NFATRE) (pGL4.30; Promega) were used to assess Gs- and Gq-coupled signaling, respectively. The activity of each mutant is expressed as a percentage (mean ± SEM) of WT activity at maximal TSH stimulation. Welch's *t* test was used for comparisons of the activities.

Protein expression levels were evaluated by Western blotting with the anti-TSHR antibody (clone 2C11; Serotec Ltd., Oxford, UK), whereas subcellular localization was studied by immunofluorescence with the anti-HA antibody (clone 3F10; Roche Applied Science, Indianapolis, IN).

Results

Characteristics of the subjects

We enrolled 24 Japanese CH patients with high RAIU. Characteristics of the subjects are shown in Supplemental Table 1. Majority of subjects had permanent CH, although three had transient CH. The thyroid size of the subjects was variable, ranging from hypoplasia (−3.1 SD) to marked goiter (+7.0 SD). (In subjects with thyroid hypoplasia, the effect of overexpression of sodium iodine symporter might be stronger than that of decreased thyroid mass.) Four subjects (17%) had goiter. Two subjects had the organification defect (*i.e.* high perchlorate discharge rate), and another had thyroglobulin (Tg) deficiency.

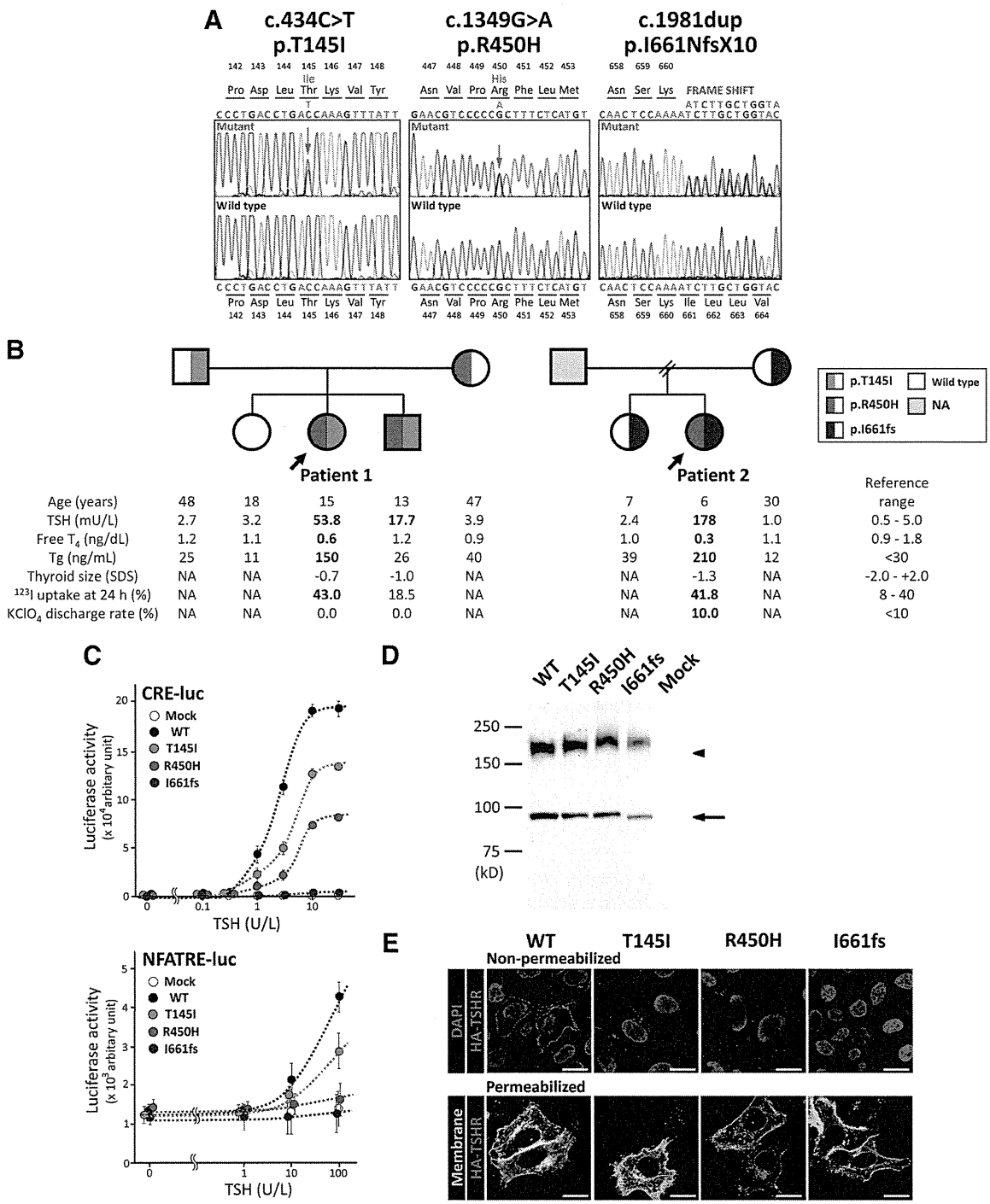
Mutation detection

We found one recurrent (c.1349G>A, p.R450H) (10) and two novel (c.434C>T, p.T145I; c.1981dup, p.I661NfsX10) *TSHR* mutations in two subjects (Fig. 1A). These three mutations were absent in the 100 control individuals. The two mutation carriers were compound heterozygote (p.[T145I]+[R450H] and p.[R450H]+[I661NfsX10]), and had no mutation in six genes implicated in thyroid dyshormonogenesis, namely *TG, TPO, SLC26A4, DUOX2, DUOXA2,* and *IYD* (data not shown).

Clinical phenotypes

The two mutation carriers were born at term from nonconsanguineous Japanese parents and were diagnosed as having CH in the frame of the newborn screening program. No family history of thyroid diseases was reported. They have been received levothyroxine replacement throughout the follow-up period, except for transient discontinuation for the thyroid status reevaluation. Genetic and clinical information of the two subjects and the members of their family is summarized in Fig. 1B. Detailed clinical information is available in the Supplemental Data.

Patient 1 (a 15 yr old girl; p.[T145I]+[R450H]) had overt hypothyroidism [TSH 53.8 mU/liter (reference 0.5–5.0); free T_4 0.6 ng/dl (reference 0.9–1.8)] with a normal-sized thyroid (−0.7 SD) when she was reevaluated at age 15



Age (years)	48	18	15	13	47	7	6	30	Reference range
TSH (mU/L)	2.7	3.2	**53.8**	**17.7**	3.9	2.4	**178**	1.0	0.5 - 5.0
Free T_4 (ng/dL)	1.2	1.1	**0.6**	1.2	0.9	1.0	**0.3**	1.1	0.9 - 1.8
Tg (ng/mL)	25	11	**150**	26	40	39	**210**	12	<30
Thyroid size (SDS)	NA	NA	-0.7	-1.0	NA	NA	-1.3	NA	-2.0 - +2.0
^{123}I uptake at 24 h (%)	NA	NA	**43.0**	18.5	NA	NA	**41.8**	NA	8 - 40
$KClO_4$ discharge rate (%)	NA	NA	0.0	0.0	NA	NA	**10.0**	NA	<10

FIG. 1. Identification and functional characterization of three *TSHR* mutations. A, Partial sequences of PCR products of subjects are shown. The heterozygous substitution of isoleucine in place of Thr145 and histidine in place of Arg450 are indicated by *arrows*. A single-base duplication in position 1981 (c.1981dup) leading to a frame shift (I661NfsX10) is also shown. B, Pedigree of patient 1 and patient 2 are shown with clinical parameters. Test results and other pertinent data are aligned with each *symbol*. Values outside the normal range are typed in *bold face*. NA, Not available. C, TSH-stimulated activation of Gs-coupled signaling (*left panel*) and Gq-coupled signaling (*right panel*) was studied with the use of the CRE-luciferase reporter and the NFATRE-luciferase reporter construct, respectively. HEK293 cells were transfected with each TSHR expression vector (WT or mutant), were incubated with various concentrations of bovine TSH, and were subject to the luciferase assay. Data are representative of three independent experiments (each performed in quadruplicate) with similar results. Values are mean ± SEM. The three mutants showed varying degrees of loss of TSH-dependent CRE and NFATRE activation. Note that R450H-TSHR could activate CRE partially but failed to activate NFATRE. D, Western blotting analysis showed that protein expression levels of each mutant were comparable with that of WT. The *arrow* and *arrowhead* indicate monomer and dimer, respectively. E, Subcellular localization analyses using HA-tagged TSHR constructs (WT or mutant). T145I-TSHR and R450H-TSHR showed attenuated but detectable membrane expression, whereas membrane expression of I661fs-TSHR was negligible. *Bars*, 20 μm.

yr. ^{123}I uptake was high (43.0% at 24 h, reference 8–40), but the perchlorate test was negative (discharge rate 0%, reference < 10). Her younger brother (a 13-yr-old boy), who had a negative result in newborn screening of CH, also had compound heterozygous mutations (Fig. 1B). At this point, he had subclinical hypothyroidism (TSH 17.7 mU/liter; free T_4 1.2 ng/dl) with normal ^{123}I uptake (18.5% at 24 h).

Patient 2 (an 8-yr-old girl; p.[R450H]+[I661NfsX10]) was shown to have overt CH (TSH 178 mU/liter, free T_4 0.3 ng/dl, Tg 210 ng/ml) when we reevaluated her thyroid status at age 5 yr. Ultrasonography demonstrated a normal-sized thyroid (−1.3 SD), which showed increased blood flow resembling Graves' disease (data not shown). ^{123}I uptake was slightly high (41.8% at 24 h), and the perchlorate discharge rate was also slightly high (10.0%).

Functional characterization of the mutant TSHR

We evaluated the capacity of TSHR (WT, T145I, R450H, and I661fs) to activate the G protein-coupled pathways (Gs or Gq) by measuring TSH-dependent activation of the downstream effectors (CRE and NFATRE for Gs and Gq, respectively) in HEK293 cells. As for Gs-coupled signaling, T145I-TSHR and R450H-TSHR showed partial activities [69 ± 2% ($P = 0.001$) and 42 ± 1% ($P < 0.001$), respectively], whereas I661fs-TSHR was nonfunctional (Fig. 1C). Evaluation of Gq-coupled signaling showed a partial activity of T145I-TSHR [51 ± 16% ($P < 0.05$)] and abrogated activities of R450H-TSHR and I661fs-TSHR [8 ± 15% ($P < 0.01$) and −5 ± 16% ($P < 0.01$), respectively]) (Fig. 1C). Western blotting showed no significant difference in protein expression levels between WT and the three mutants (Fig. 1D). Subcellular localization analyses demonstrated that I661fs-TSHR were entrapped in the intracellular compartment and were poorly expressed in the plasma membrane (Fig. 1E). T145I-TSHR and R450H-TSHR also showed attenuated membrane expression.

Discussion

In the present study, one recurrent (p.R450H) and two novel (p.T145I and p.I661NfsX10) *TSHR* mutations were identified. Although T145I-TSHR and I661fs-TSHR showed balanced deterioration of coupling to Gs and Gq that was mild and severe, respectively, R450H-TSHR showed the Gq-dominant coupling defect. The Arg450 residue is located in the first intracellular loop (ICL1) of TSHR, in which the α-subunit of Gq is suggested to make contacts (11). A systematic mutagenesis study showing that the Gq-dominant coupling defect is a common feature of mutations in ICL1 (11) also suggests the essential role of ICL1 in Gq coupling. The Gq-dominant coupling defect has also been observed in the p.L653V mutation, which is located in the third extracellular loop and produces the high RAIU phenotype (6).

TSH stimulates thyroidal iodine uptake. Hence, increased iodine uptake in the TSH resistant state is apparently discrepant. We presume that differential control of cellular processes by the two G proteins reconciles the discrepancy. Recently a study using thyroid-specific Gq/G11-knockout mice has shown that Gq mediates TSH stimulated cell proliferation and Tg iodination but does not mediate iodine uptake (12). On the contrary, Gs can mediate all the three processes (13–15). These data suggest a model in which cell proliferation and Tg iodination are controlled by Gs and Gq (dual control), whereas iodine uptake is solely controlled by Gs (single control) (Fig. 2A). In a thyroid carrying the *TSHR* mutation that affects coupling to both Gs and Gq, the patient will have low RAIU and small thyroid if the resistance is severe (uncompensated TSH resistance; Fig. 2C, *left panel*), or the patient will have normal RAIU and normal thyroid size (compensated TSH resistance; Fig. 2C, *middle panel*) if the resistance is mild. In a thyroid carrying the mutation with the Gq-dominant coupling defect, RAIU would be elevated via the enhanced Gs-coupled signaling (Fig. 2C, *right panel*). Deleterious effects of the mutation on thyroid size and Tg iodination would be determined by the balance between the enhanced Gs-coupled signaling and deteriorated Gq-coupled signaling and thus could be variable. In our patients, thyroid size was slightly small but in the normal range. As for Tg iodination, the perchlorate discharge rate of the patients was variable.

Previously reported mutation carriers that were homozygous or compound heterozygous for p.R450H did not necessarily show the high RAIU phenotype (Supplemental Fig. 1) (Refs. 8, 10, and 16 and our unpublished observations). Moreover, the younger brother of patient 2 (p.[T145I]+[R450H]) had normal ^{123}I uptake. The phenotypic difference between these patients and our two probands is likely explained by the difference in serum TSH levels (14.3–30.1 mU/liter in the former group; 53.8 and 178 mU/liter in the latter group) because it is reasonable to assume that higher levels of compensatory TSH rise are required for clinically recognizable RAIU elevation.

The major limitation of this study is the ethnic difference in genotype distribution. The p.R450H mutation, which has the Gq-dominant coupling defect, is especially common in Japan (8). Therefore, investigations in other ethnic groups are needed to obtain more generalizable frequency data.

To conclude, we identified two patients with molecularly confirmed TSH resistance that presented discrep-

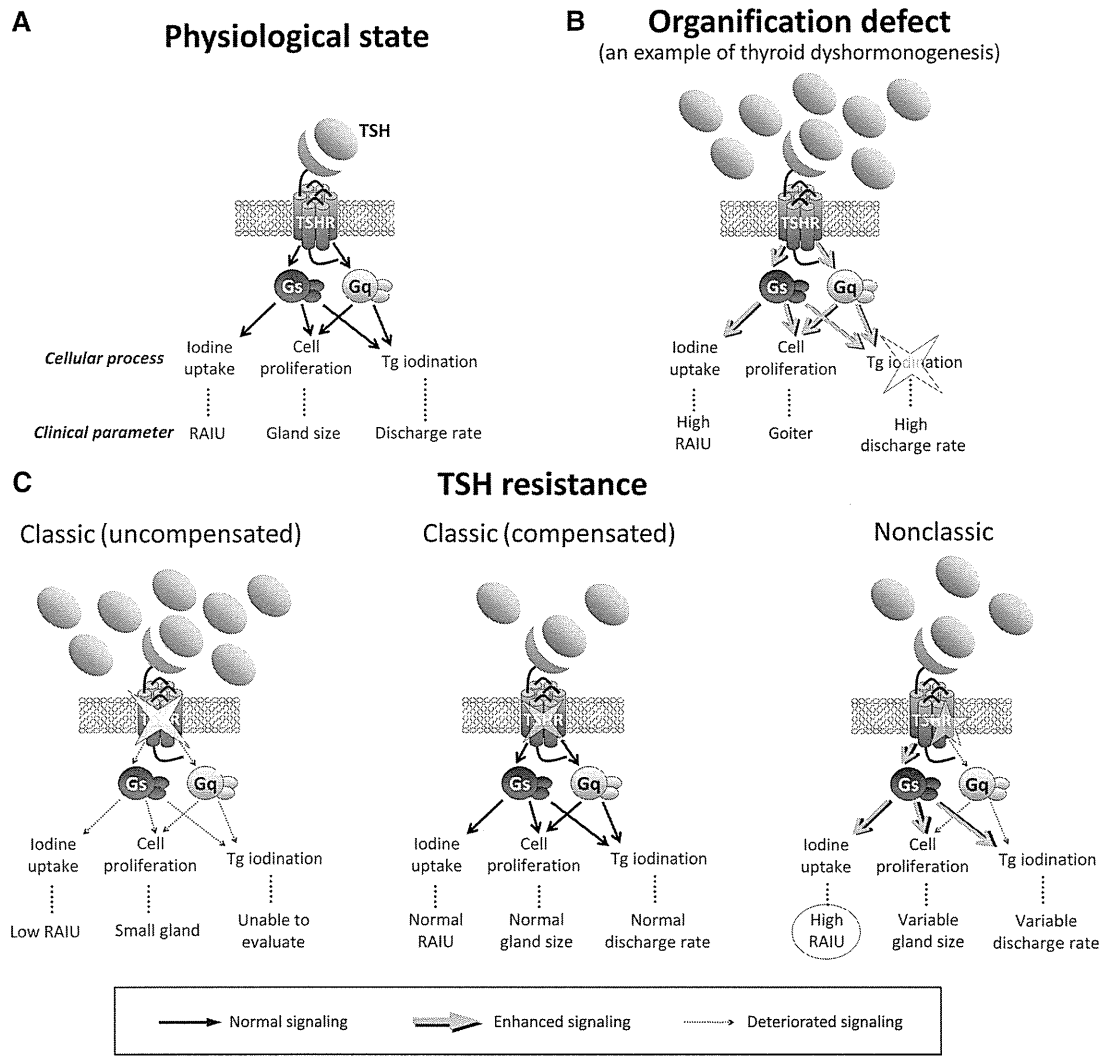


FIG. 2. Schematic diagrams of the G protein-coupled signaling pathways and downstream cellular responses in the thyroid cells. A, In the physiologic state, ligand-bound TSHR couples to both Gs and Gq and activates several downstream cellular processes. The status of thyroidal iodine uptake, cell proliferation, and Tg iodination can be clinically assessed by RAIU, thyroid gland size, and iodine discharge after perchlorate administration, respectively. Note that cell proliferation and Tg iodination are controlled by both Gs and Gq (dual control), whereas iodine uptake is solely controlled by Gs (single control). B, In the organification defect (primary defect of Tg iodination; an example of thyroid dyshormonogenesis), increased TSH stimulates both Gs- and Gq-coupled pathways, resulting in high RAIU and goiter. C, Three panels show the intracellular signaling of classic and nonclassic TSH resistance. *Left panel*, Uncompensated classic TSH resistance. Both Gs- and Gq-coupled pathways are deteriorated. Patients show the clinical features of thyroid dysgenesis (*i.e.* low RAIU and a small thyroid). *Middle panel*, Compensated classic TSH resistance. Both Gs- and Gq-coupled pathways are compensated by increased TSH. Patients show the phenotype that is recognized as idiopathic congenital hypothyroidism (*i.e.* normal RAIU, a normal-sized thyroid, and normal perchlorate discharge rate). *Right panel*, Nonclassic TSH resistance, in which Gq coupling is dominantly affected. Enhanced Gs-coupled signaling causes high RAIU as seen in thyroid dyshormonogenesis. Deleterious effects of mutations on thyroid size and Tg iodination would vary, depending on the balance between enhanced Gs-coupled signaling and deteriorated Gq-coupled signaling.

antly high RAIU. Our data support the idea that the phenotypic spectrum of TSH resistance is even broader than previously thought. We propose to term this dyshormonogenesis-like variant of TSH resistance as nonclassic TSH resistance, which is observed in 8% of Japanese CH patients with high RAIU. We stress that a patient with high RAIU should be considered for nonclassic TSH resistance as well as other forms of thyroid dyshormonogenesis, especially if the patient lacks goiter.

Acknowledgments

We thank Professor T. Takahashi for fruitful discussion.

Address all correspondence and requests for reprints 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.

This work was supported by a Grant-in-Aid for Young Scientists (B) (21791006) from the Japan Society for the Promotion of Science, a Grant-in-Aid for Scientific Research (C)

(20591232) from the Japan Society for the Promotion of Science, and a Grant for Child Health and Development (20C-2) from the Ministry of Health, Labor, and Welfare of Japan.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Refetoff S** 2003 Resistance to thyrotropin. J Endocrinol Invest 26: 770–779
2. **Sunthornthepvarakui T, Gottschalk ME, Hayashi Y, Refetoff S** 1995 Brief report: resistance to thyrotropin caused by mutations in the thyrotropin-receptor gene. N Engl J Med 332:155–160
3. **Persani L, Calebiro D, Cordella D, Weber G, Gelmini G, Libri D, de Filippis T, Bonomi M** 2010 Genetics and phenomics of hypothyroidism due to TSH resistance. Mol Cell Endocrinol 322:72–82
4. **Tiosano D, Pannain S, Vassart G, Parma J, Gershoni-Baruch R, Mandel H, Lotan R, Zaharan Y, Pery M, Weiss RE, Refetoff S, Hochberg Z** 1999 The hypothyroidism in an inbred kindred with congenital thyroid hormone and glucocorticoid deficiency is due to a mutation producing a truncated thyrotropin receptor. Thyroid 9:887–894
5. **Abramowicz MJ, Duprez L, Parma J, Vassart G, Heinrichs C** 1997 Familial congenital hypothyroidism due to inactivating mutation of the thyrotropin receptor causing profound hypoplasia of the thyroid gland. J Clin Invest 99:3018–3024
6. **Grasberger H, Van Sande J, Hag-Dahood Mahameed A, Tenenbaum-Rakover Y, Refetoff S** 2007 A familial thyrotropin (TSH) receptor mutation provides *in vivo* evidence that the inositol phosphates/Ca2+ cascade mediates TSH action on thyroid hormone synthesis. J Clin Endocrinol Metab 92:2816–2820
7. **Yasumoto M, Inoue H, Ohashi I, Shibuya H, Onishi T** 2004 Simple new technique for sonographic measurement of the thyroid in neonates and small children. J Clin Ultrasound 32:82–85
8. **Narumi S, Muroya K, Abe Y, Yasui M, Asakura Y, Adachi M, Hasegawa T** 2009 TSHR mutations as a cause of congenital hypothyroidism in Japan: a population-based genetic epidemiology study. J Clin Endocrinol Metab 94:1317–1323
9. **Narumi S, Muroya K, Asakura Y, Adachi M, Hasegawa T** 2010 Transcription factor mutations and congenital hypothyroidism: systematic genetic screening of a population-based cohort of Japanese patients. J Clin Endocrinol Metab 95:1981–1985
10. **Nagashima T, Murakami M, Onigata K, Morimura T, Nagashima K, Mori M, Morikawa A** 2001 Novel inactivating missense mutations in the thyrotropin receptor gene in Japanese children with resistance to thyrotropin. Thyroid 11:551–559
11. **Kleinau G, Jaeschke H, Worth CL, Mueller S, Gonzalez J, Paschke R, Krause G** 2010 Principles and determinants of G-protein coupling by the rhodopsin-like thyrotropin receptor. PLoS One 5:e9745
12. **Kero J, Ahmed K, Wettschureck N, Tunaru S, Wintermantel T, Greiner E, Schütz G, Offermanns S** 2007 Thyrocyte-specific Gq/G11 deficiency impairs thyroid function and prevents goiter development. J Clin Invest 117:2399–2407
13. **Saito T, Endo T, Kawaguchi A, Ikeda M, Nakazato M, Kogai T, Onaya T** 1997 Increased expression of the Na+/I− symporter in cultured human thyroid cells exposed to thyrotropin and in Graves' thyroid tissue. J Clin Endocrinol Metab 82:3331–3336
14. **Dumont JE, Lamy F, Roger P, Maenhaut C** 1992 Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. Physiol Rev 72:667–697
15. **Rigutto S, Hoste C, Grasberger H, Milenkovic M, Communi D, Dumont JE, Corvilain B, Miot F, De Deken X** 2009 Activation of dual oxidases Duox1 and Duox2: differential regulation mediated by camp-dependent protein kinase and protein kinase C-dependent phosphorylation. J Biol Chem 284:6725–6734
16. **Shibayama K, Ohyama Y, Hishinuma A, Yokota Y, Kazahari K, Kazahari M, Ieiri T, Matsuura N** 2005 Subclinical hypothyroidism caused by a mutation of the thyrotropin receptor gene. Pediatr Int 47:105–108

RESEARCH LETTER

AMERICAN JOURNAL OF medical genetics PART A

A Family of Pseudohypoparathyroidism Type Ia With an 850-kb Submicroscopic Deletion Encompassing the Whole *GNAS* Locus

Toshikatsu Mitsui,[1] Keisuke Nagasaki,[2] Masaki Takagi,[1] Satoshi Narumi,[1] Tomohiro Ishii,[1] and Tomonobu Hasegawa[1]*

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

[2]Division of Pediatrics, Department of Homeostatic Regulation and Development, Niigata University Graduate School of Medicine and Dental Sciences, Niigata, Japan

Received 30 September 2011; Accepted 26 October 2011

TO THE EDITOR:

Pseudohypoparathyroidism (PHP) is a heterogeneous genetic disorder that is hallmarked by end-organ resistance to parathyroid hormone (PTH). PHP type Ia (PHP-Ia) is characterized by the presence of Albright hereditary osteodystrophy (AHO), which includes short stature, obesity, round face, brachydactyly, subcutaneous calcification, and intellectual disability. Additionally, mild resistance to other hormones (e.g., thyroid stimulating hormone, growth hormone releasing hormone) can be observed [Bastepe and Juppner 2003]. PHP-Ia is usually caused by a point mutation in *GNAS*, which encodes the α subunit of Gs protein (Gsα) [Weinstein et al., 2001]. The *GNAS* mutations leading to PHP-Ia are all heterozygous and that all are located on the maternal allele. Gsα expression appears to occur in few tissues from only the maternal allele, thus only a handful of G protein-coupled receptors fails to mediate the actions of their cognate hormones, if a mutation is present on the maternal *GNAS* allele. Whereas the mutations inherited from the paternal allele leads to pseudo-pseudohypoparathyroidism (PPHP) [Wilson et al., 1994], which shows AHO without PTH resistance [Albright et al., 1952]. This peculiar pattern of inheritance (i.e., parent-of-origin effect) can be explained by tissue- and parent-specific imprinting of *GNAS* [Liu et al., 2005]. In most tissues [Campbell et al., 1994; Hayward et al., 1998], *GNAS* is expressed biallelically, whereas a paternal allele is silenced and only maternal allele expresses Gsα in several tissues including renal proximal tubules [Yu et al., 2000]. Therefore, a maternally transmitted *GNAS* mutation leads to PTH resistance (i.e., PHP-Ia), but a paternally transmitted mutation does not (i.e., PPHP). *GNAS* haploinsufficiency in tissues with biallelic *GNAS* expression causes AHO. Thus, AHO is observed irrespective of the origin of the mutation. About 70% of patients with PHP-Ia/PPHP have point mutations in *GNAS* [Ahrens et al., 2001]. Thus, molecular pathogenesis of 30% of patients remains unclear. Here, we report on the identification of the first family with PHP-Ia/PPHP harboring a submicroscopic deletion involving the whole *GNAS* locus.

Patients were a 17-year-old girl (Patient 1) and a boy (Patient 2), who were dizygotic twins. The details of clinical information have been described previously [Nagasaki et al., 2005]. The pedigree and related clinical data are shown in Figure 1. Patient 1, a Japanese girl, was first referred to us presenting subcutaneous calcification on her back at age 10 years. She had AHO: short stature (adult height, 144 cm; −2.5 SD), obesity (BMI 30.0) [Matsuzawa et al., 2002], round face, brachydactyly (shortening of fourth and fifth metacarpal bones), and subcutaneous calcification on her left ankle and back. She also had nail hypoplasia, dental root defects, and oligomenorrhea, but had no intellectual disability. She had compensated PTH resistance (an increased PTH level with a normal serum Ca level). Patient 2 had AHO, including short stature (adult height, 155 cm; −2.6 SD), equivocal round face, and subcutaneous calcification on his left hand. He had neither of brachydactyly, obesity (BMI 24.8) nor intellectual disability. He had hypocalcemia due to PTH resistance. The mother of Patients 1 and 2 had AHO: short stature (148 cm; −2.0 SD), obesity (BMI 26.5), and round face. She had neither brachydactyly nor intellectual disability. She had normal serum levels of Ca and PTH.

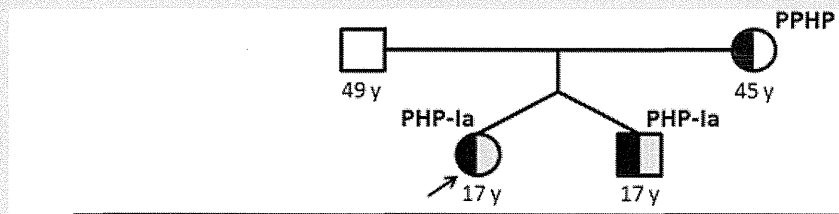
Based on clinical findings, we diagnosed the two patients and their mother as having PHP-Ia and PPHP, respectively. We suspected that the two PHP-Ia patients had a *GNAS* mutation in their

How to Cite this Article:
Mitsui T, Nagasaki K, Takagi M, Narumi S, Ishii T, Hasegawa T. 2012. A family of pseudohypoparathyroidism type Ia with an 850-kb submicroscopic deletion encompassing the whole *GNAS* locus.
Am J Med Genet Part A 158A:261–264.

Additional supporting information may be found in the online version of this article.

*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@a6.keio.jp

Published online 2 December 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.34393



		Father	Patient 1	Patient 2	Mother
AHO	Height (cm) (SDS)	175 (+0.8)	144 (-2.6)	155 (-2.6)	148 (-2.0)
	BMI	24.5	30.0	24.8	26.5
	Round face	Absent	Present	Equivocal	Present
	Brachydactyly	Absent	Present	Absent	Absent
	Subcutaneous calcification	Absent	Present	Present	Absent
	Mental retardation	Absent	Absent	Absent	Absent
PTH resist	Ca (mg/dl) [a]	8.9	8.9	7.0	9.5
	PTH (pg/ml) [b]	44	152	377	26
	Other findings		Oligomenorrhea Nail hypoplasia Dental root defects	Intracranial calcification [c]	Oligomenorrhea

FIG. 1. The pedigree and clinical data. Test results and other pertinent data are aligned with each symbol. Phenotypes consistent with Albright hereditary osteodystrophy (AHO) are shown in black, and data indicating parathyroid hormone (PTH) resistance are shown in gray. Note that both AHO phenotypes and severity of PTH resistance were variable between the twins. The mother had partial features of AHO without PTH resistance. AHO, Albright's hereditary osteodystrophy; BMI, body mass index; NA, not available; PHP-Ia, pseudohypoparathyroidism type Ia; PPHP, pseudo-pseudohypoparathyroidism; PTH, parathyroid hormone; SDS, standard deviation score. [a]Reference range, 8.5–10.2. [b]Reference range, 10–65. [c]Intracranial calcification was evaluated by computed tomography.

maternal allele. We first sought a point mutation using PCR-based sequencing, but found no mutation. Also heterozygous SNPs were not identified when sequencing the *GNAS* exons. We then considered a deletion/duplication, and carried out Multiplex ligation-dependent probe amplification (MLPA; MRC-Holland, Amsterdam, The Netherlands) analyses. As a result, we found that all 22 signals derived from *GNAS* were attenuated in the patients and the mother (data not shown). The deletion was confirmed by oligonucleotide aCGH analyses (SurePrintG3 180K; Agilent Technologies, Santa Clara, CA), which revealed an 850-kb deletion at 20q13.32 encompassing the whole *GNAS* locus and 8 other genes (i.e., *NPEPL1*, *TH1L*, *CTSZ*, *TUBB1*, *ATP5E*, *SLMQ2*, *ZNF831*, and *EDN3*; Fig. 2). Haplotype analyses using two markers flanking *GNAS* (D20S100 and D20S171) showed that the patients had the identical haplotypes, indicating that they shared the common maternal and paternal alleles (Supporting Information online Supplementary eFig. 1.). Methylation-specific MLPA showed methylation defects at *NESPAS*, GNASXL, and GNAS exon A/B in Patients 1 and 2. On the other hand methylation defect at NESP55 in the mother (Supporting Information online Supplementary eFig. 2).

In this study, we identified the first PHP-Ia patients with a submicroscopic whole deletion of *GNAS*, which was maternally inherited. A subset of "*GNAS* mutation-negative" PHP-Ia/PPHP patients might have a submicroscopic *GNAS* deletion, because such mutations cannot be detected by standard PCR-based sequencing. To date, four unrelated PHP-Ia/PPHP patients with a microscopically detectable de novo deletion encompassing *GNAS* have been described [Aldred et al., 2002; Genevieve et al., 2005]. All four patients had multiple atypical manifestations, such as severe pre- and postnatal growth retardation, intractable feeding difficulties, microcephaly, hypotonia, and facial anomalies. The complex phenotypes, likely due to larger size of deletions (at least 2 Mb), made it difficult to assume the phenotype of *GNAS* haploinsufficiency. Contrastingly, presented two patients and their mother all fit the known phenotypic spectrum of PHP-Ia/PPHP, and had no atypical manifestations. This unique observation provides following three implications. First, heterozygous deletion of 8 adjacent genes (*NPEPL1–EDN3*) causes no recognizable phenotype, suggesting that these 8 are recessive loci and not differentially methylated. Second, the effects of intragenic point mutations are likely as deleterious as haploinsufficiency. Third, non-coding regions of *GNAS*, including several differentially methylated regions (e.g., NESP, *GNASAS*, GNASXL), would have no *cis*-effect on gene(s) other than *GNAS*. Contrasting to de novo occurrence of deletions in previous four patients, the deletions of the present patients were

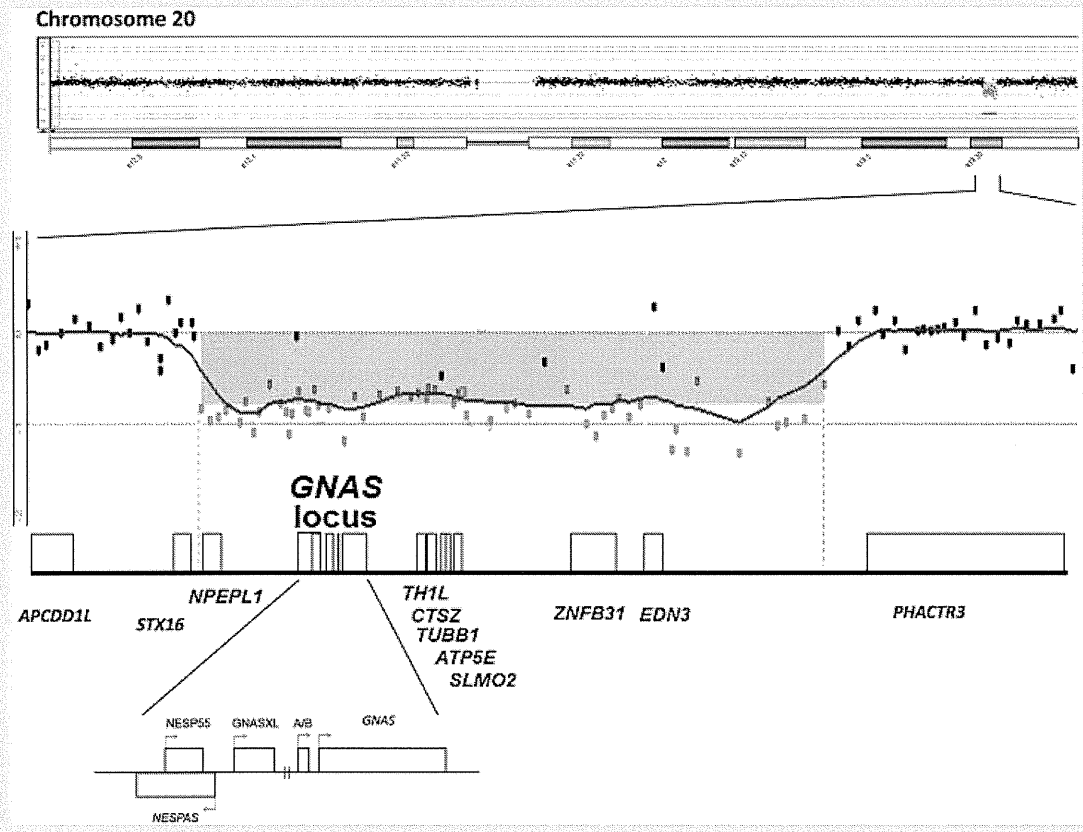


FIG. 2. The 850-kb deletion encompassing the whole *GNAS* locus revealed by oligonucleotide array comparative genomic hybridization. Each dot represents ratio of Log_2 [Cy5/Cy3]. The horizontal axis corresponds to chromosomal location and the vertical axis corresponds to log ratio. The gray area indicates the deletion including the *GNAS* locus and 8 other genes. The *GNAS* locus consists of 13 exons and 4 promoters, such as NESP55, *NESPAS*, GNASXL, and GNAS exon A/B. [Color figure can be seen in the online version of this article, available at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4833]

inherited from a mother to her two offspring. This circumstance enabled us to compare the phenotypes among the family members. Of interest, expressivity of both AHO and PTH resistance were variable between the patients: one had 5 features of AHO with compensated PTH resistance, whereas the other had only 2 AHO features but had overt PTH resistance. Considering that these patients had pure *GNAS* haploinsufficiency and the common paternal allele, other factor(s) than the genomic *GNAS* abnormality might contribute to phenotypic variability. Possible factors include nutrition (e.g., calcium, phosphate, and vitamin D), other genetic factors (e.g., Gs/cAMP pathway, calcium metabolism), imprinting status of the paternal *GNAS* locus, and a stochastic mechanism.

In conclusion, we identified the first PHP-Ia patients harboring a submicroscopic whole deletion of *GNAS*. This type of mutation is undetectable by standard PCR-based sequencing, but can be captured by MLPA and aCGH. We stress that PHP-Ia patients that have no apparent mutation in the protein-coding regions of *GNAS* should be considered for a submicroscopic structural mutation.

REFERENCES

Ahrens W, Hiort O, Staedt P, Kirschner T, Marschke C, Kruse K. 2001. Analysis of the GNAS1 gene in Albright's hereditary osteodystrophy. J Clin Endocrinol Metab 86:4630–4634.

Albright F, Forbes AP, Henneman PH. 1952. Pseudo-pseudohypoparathyroidism. Trans Assoc Am Physicians 65:337–350.

Aldred MA, Aftimos S, Hall C, Waters KS, Thakker RV, Trembath RC, Brueton L. 2002. Constitutional deletion of chromosome 20q in two patients affected with Albright hereditary osteodystrophy. Am J Med Genet 113:167–172.

Bastepe M, Juppner H. 2003. Editorial: Pseudohypoparathyroidism and mechanisms of resistance toward multiple hormones: Molecular evidence to clinical presentation. J Clin Endocrinol Metab 88:4055–4058.

Campbell R, Gosden CM, Bonthron DT. 1994. Parental origin of transcription from the human GNAS1 gene. J Med Genet 31:607–614.

Genevieve D, Sanlaville D, Faivre L, Kottler ML, Jambou M, Gosset P, Boustani-Samara D, Pinto G, Ozilou C, Abeguile G, Munnich A, Romana S, Raoul O, Cormier-Daire V, Vekemans M. 2005. Paternal deletion of the GNAS imprinted locus (including Gnasxl) in two girls presenting with

severe pre- and post-natal growth retardation and intractable feeding difficulties. Eur J Hum Genet 13:1033–1039.

Hayward BE, Kamiya M, Strain L, Moran V, Campbell R, Hayashizaki Y, Bonthron DT. 1998. The human GNAS1 gene is imprinted and encodes distinct paternally and biallelically expressed G proteins. Proc Natl Acad Sci USA 95:10038–10043.

Liu J, Chen M, Deng C, Bourc'his D, Nealon JG, Erlichman B, Bestor TH, Weinstein LS. 2005. Identification of the control region for tissue-specific imprinting of the stimulatory G protein alpha-subunit. Proc Natl Acad Sci USA 102:5513–5518.

Matsuzawa Y, Nakamura T, Takahashi M, Miwa R, Inoue S, Ikeda Y, Ohno M, Sakata T, Fukagawa K, Saitoh Y, Sato Y, Shirai K, Miyazaki S, Tokunaga K, Yamanouchi K, Takahashi M, Shibasaki T, Nagai M. 2002. New criteria for 'obesity disease' in Japan. Circ J 66:987–992.

Nagasaki K, Shimomura Y, Suyama T, Magara S, Ogawa Y, Hiura M, Kikuchi T, Uchiyama M. 2005. Two cases of pseudohypoparathyroidism type Ia in duozygotic twins with different phenotypes. Clin Pediatr Endocrinol 14:39–44.

Weinstein LS, Yu S, Warner DR, Liu J. 2001. Endocrine manifestations of stimulatory G protein alpha-subunit mutations and the role of genomic imprinting. Endocr Rev 22:675–705.

Wilson LC, Oude Luttikhuis ME, Clayton PT, Fraser WD, Trembath RC. 1994. Parental origin of Gs alpha gene mutations in Albright's hereditary osteodystrophy. J Med Genet 31:835–839.

Yu S, Gavrilova O, Chen H, Lee R, Liu J, Pacak K, Parlow AF, Quon MJ, Reitman ML, Weinstein LS. 2000. Paternal versus maternal transmission of a stimulatory G-protein alpha subunit knockout produces opposite effects on energy metabolism. J Clin Invest 105:615–623.

特　集　最近話題のホルモン受容体異常症

GHRH受容体異常症

曽根田 明子*　安達 昌功*

1 疾患概念

下垂体前葉からの成長ホルモン(GH)分泌は，主として視床下部のGH放出ホルモン(GHRH)およびソマトスタチンにより制御されている．GHRH受容体異常症とは，下垂体ソマトトロフに発現するGHRH受容体の先天的異常のため生じるGH単独欠損症(isolated Growth Hormone deficiency : IGHD)をいう．

IGHDは，出生後に始まる成長障害を主徴とし，その頻度は4,000～10,000出生に1人と報告されている[1-3]．このうちの多くは特発性であり，また一部は視床下部～下垂体領域における虚血・炎症・腫瘍等の後天的要因によるものである．遺伝子異常による遺伝性IGHDは稀であり，報告により5～30％とされている[4]．

遺伝性IGHDは臨床的特徴や遺伝形式等により4つのタイプに分類されている(表1)．GHRH受容体異常症はタイプIBに分類され，常染色体劣性の遺伝形式を呈する．

2 GHRH受容体

GHRH受容体はG蛋白共役受容体(G-protein coupling receptor : GPCR)の一つであり，423のアミノ酸より構成されている．GPCRファミリーの中のBグループⅢに属し，細胞外N末端領域，7回膜貫通領域，および細胞内C末端領域を有する．リガンドであるGHRHが受容体に結合することで，セカンドメッセンジャーであるcAMPの合成が促され，プロテインキナーゼA経路の活性化を経てGHが分泌される．また，リガンドの結合によるMAPキナーゼ活性化も報告されている[5,6]．GHRH受容体遺伝子(*GHRHR*)は常染色体7p14に存在し，13個のエクソンより成り立っている[7]．

3 GHRH受容体遺伝子(*GHRHR*)変異の報告例

最初のGHRH受容体遺伝子異常として，1993年に遺伝性GH欠損マウスモデルであるLittleマウスにおけるホモ接合体変異(D60G)が見いだされた[8,9]．

ヒトでは，1990年代後半に2つの変異が低身長の大家系で発見されている．1つは，インド[10]，パキスタン[11]，スリランカ[12]から報告されたエクソン3のE72X変異であり，後にこれらはリンケージ解析で共通の祖先に由来することが判明した[13]．他方は，ブラジル北東部の大家族例から報告された，スプライス変異(IVS1+1g>a)である[14]．

現在までに，プロモーター領域の変異，ミスセンス変異，ナンセンス変異，スプライス異常，微小欠失を合わせ，約20の*GHRHR*変異が報告されている(表2)．多くは前述のように一定の地域に限局した血族婚のある家系からの報告であり，孤

* 神奈川県立こども医療センター　内分泌代謝科
Akiko Soneda : GHRH receptor mutation.
Department of Endocrinology & Metabolism, Kanagawa Children's Medical Center.

表1　遺伝性IGHDの分類

タイプ	遺伝形式	原因遺伝子	GH分泌	特徴
I A	AR	*GH1* 欠失/*GH1* 変異	欠損	重度の低身長、治療による抗GH抗体が出現が見られる
I B	AR	*GH1* 変異/*GHRHR* 変異	低下	低身長、GH治療に対する反応は良好
II	AD	*GH1* 変異	低下	低身長、GH治療に対する反応は良好
III	X連鎖	*BTK* 変異/未同定	低下	*BTK* 変異は低ガンマグロブリン血症を伴う

AD：常染色体優性，AR：常染色体劣性

表2　既報のGHRH受容体異常症のまとめ

変異	国・地域名	性別	報告時の年齢	身長SDS	負荷試験でのGH頂値 (ng/mL) GHRH	負荷試験でのGH頂値 (ng/mL) インスリン	IGF-1 (ng/mL)	下垂体低形成 (検査時年齢)
-124A>C / K329F	不明	M	4y	-4.6	1.6	1.2		+(4y)
IVS1+1G>A ホモ	ブラジル	M9, F13	5y ~ 20y	-2.7 ~ -8.4	0.63 ± 0.61	"flat"	6.7 ± 2.8	ND
IVS1+1G>A / IVS4-2A>G	ブラジル	F	9y	-6.2		1.5		+(9y)
IVS1+1G>A / E382E	ブラジル	F	7y	-3.3		0.1		+(16y)
IVS1+2T>G ホモ	モロッコ	M1, F1	9y, 11y	-5.4 ~ -6.6		0.1 ~ 4.2	17.4 ~ 21	+(9y), +(11y)
Q43X / IVS3+1G>A	不明	M2	8y, 11y	-5.5	<0.05		16 ~ 20	+(18y), -(20y)
IVS2+3A>G / G136V	日本	F	2y	-3.1	3.93	2.81	7	-(2y)
E72X ホモ	多数報告例あり							
E72X / R161W	アジア	M1, F1	4y, 5y	-4.5 ~ -4.8		0.6 ~ 0.7	<-2.5SD	-(4y), +(5y)
R94L ホモ	アジア	F	5y	-5.6		0.9	-3.0SD	+(5y)
391delG ホモ	エジプト	F3	5.9y	-5.5 ~ -6.0			<6 ~ 9	-(4y), +(5y)
H137L / del 1140-1144	不明	M1, F1	11y, 14y	-5.9 ~ -7.0		1.0		ND
L144H ホモ	スペイン	M2	3y ~ 5y	-3.3 ~ -4.0	0.41 ~ 2.0		22 ~ 40	+(5y)
	ブラジル	F2	19y ~ 26y	-7.1 ~ -7.4	<0.3	<0.3	2.5 ~ 4.6	+(19y), +(26y)
L144H / F242C	米国	M2	16y ~ 17y	-4.5 ~ -5.2		2.3 ~ 3.8		ND
A176V ホモ	パキスタン	M2	8y	-3.5 ~ -4.5	<1mC.T.			+(8y)
A222E ホモ	パキスタン	M2	2y ~ 11y	-5.2 ~ -6.8				ND
	アジア	M1, F1	2y, 3y	-2.8 ~ -3.0		0.4	<-3.5SD	+(3y)
IVS7+1G>C ホモ	モロッコ	M1, F1	14y, 16y	-5.1 ~ -7.3	"absent"		14.5 ~ 26.8	-(23y), -(25y)
IVS7-1G>A ホモ	ブラジル	M 1, F1	3y ~ 10y	-2.5 ~ -3.9	1.8 ~ 2.6		18	ND
IVS8+1G>A ホモ	中国	F3	36y ~ 56y	120 ~ 121cm		0.15	31.5	+(36y), +(42y)
W273S ホモ	アジア	F2	2y ~ 6y	-2.8 ~ -4.9		0.9	-1.9SD ~ -3.5SD	-(2y), +(6y)
E382E ホモ	日本	F2	5y, 7y	-5.2 ~ -5.6	2.0			-(5y), -(7y)
del 1121-1124 ホモ	日本	M	3y	-6.0	0.2		67.9	+(3y)
IVS12+2T>A ホモ	パキスタン	M1, F2	2y ~ 8y	-2.5 ~ -3.6	0.6 ~ 1.0		11, 49	+(3y), -(6y), -(8y)

文献19より一部改編
M 男性，F 女性，ND not done.

発例の報告は少ない．IGHDを呈し，正常位置に下垂体が観察された224名の遺伝子解析を行ったイギリスの報告では，7家系・15例に*GHRHR*変異が発見されたが，いずれもnon-Caucasianで，両親の血族婚を有する家族からの症例であった[15]．また，IGHD患者89名を対象にしたオランダからの報告では*GHRHR*変異は同定されなかった[16]．

国内では4例のGHRH受容体異常症が報告され

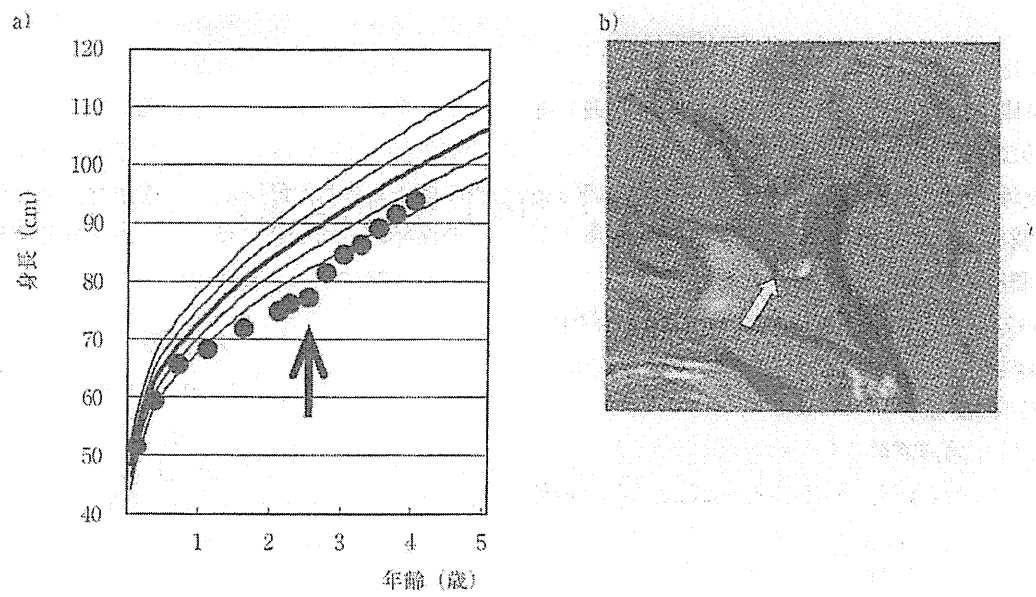


図1 a) 成長曲線．矢印はGH補充療法開始時を示す．
b) 下垂体MRI画像．

ている．堀川によるエクソン12の4塩基欠失例（del1121-1124）[17]，井上らによるE382Eの同胞例[16]，筆者らによるG136VとIVS2+3a>gとの複合ヘテロ接合体変異（後述）であり[19]，これらはいずれも血族婚を有さない症例である．井上らの検討では，IGHD 14例および特発性低身長113例において*GHRHR*解析が行われたが，E382E同胞例以外には変異は見いだされなかった[18]．

4 臨床症状

全例が重篤な低身長を呈する．子宮内での発育障害は見られず，成長障害は1歳前後から観察される．無治療の症例では-4～-8SDの低身長となる．知能発達は正常であり，GH-1遺伝子異常症で観察される新生児低血糖は見られず，また前額部突出や腹部肥満はあっても軽度である[20]．男児では小陰茎は認めない．

血液検査所見ではIGF-1が低値を示す．GH分泌刺激試験に対するGH分泌も不良であり，多くの症例は重症成長ホルモン分泌不全性低身長症の所見を示す．他の下垂体ホルモン分泌に異常は見られない．一部の症例で思春期の発来が遅れるようだが，妊孕性に問題はない．

MRI検査では多くの症例で下垂体前葉低形成が観察され，これは下垂体ソマトトロフ細胞の低形成の反映と考えられる．しかし，報告例の多くは6歳以降の症例であり，出生時または乳児期から下垂体低形成が観察されるか否かは不明である．自験例では2歳時にMRIを施行しており，この時点では下垂体低形成は明らかではなかった[19]．

GH補充療法を開始した後の成長は良好であり，GH治療によく反応する．GH抗体の出現に関する報告はなく，適切な時期に治療を行えば目標身長への到達が期待できる．

5 臨床症例

筆者らが経験したGHRH受容体異常症を紹介する[19]．

【症例】初診時2歳3カ月，女児

【経過】妊娠・分娩経過に異常なく，在胎38週に頭位・自然分娩にて出生．出生時体重3,236g，身長50.0cm，頭囲33.0cm．1歳半健診で低身長を指摘され，以後も徐々に低身長が著明となり当院へ紹介された（図1a）．精神運動発達は正常であ

った.

【既往歴】特記事項なし.

【家族歴】父 169.5cm, 母 156cm, 兄（4歳）身長 +0.5SD. 血族婚なし.

【身体所見】身長 77.2cm (−3.1SD), 体重 8.9kg（肥満度 0%), 頭囲 44.0cm (−2.1SD), 軽度の前額部突出を認める.

【内分泌検査所見】IGF-1 7ng/mL, IGFBP-3 0.41μg/mL, TSH 3.73μIU/mL, FT3 3.75pg/mL, FT4 1.16ng/dL.

GH分泌刺激試験：

インスリン負荷：GH前値 0.27ng/mL, 頂値 2.81ng/mL

アルギニン負荷：GH前値 2.05ng/mL, 頂値 3.78ng/mL

GHRH負荷：GH前値 0.64ng/mL, 頂値 3.93ng/mL

【頭部MRI】下垂体高は 3mm で正常範囲内（図 1b).

【遺伝子解析】*GH-1* に変異を認めなかったため, *GHRHR* の解析を行った. その結果, G136V と IVS2+3a>g の複合ヘテロ接合体変異を同定した. 両親の遺伝子解析の結果, G136V は父親由来, IVS2+3a>g は母親由来と判明した. G136V は GHRH 受容体の第1膜貫通領域にあり, 機能解析にて変異受容体では GHRH に対する cAMP の反応を認めなかった. また, IVS2+3a>g は mini-gene を用いたスプライスアッセイにより, 異常スプライスをきたすことが示唆された. すなわち, IVS2+3a>g により正常スプライスドナー部位の機能はほぼ完全に損なわれ, イントロン2が保持された転写産物が増加, またはより下流での cryptic splice site activation が起こりフレームシフトが起きると予想された.

【経過】GH補充療法に対する反応は良好であった（図 1a).

6 まとめ

GHRH受容体異常症は, 遺伝性IGHDタイプIBの表現型を示す稀な疾患である. 生後の早い段階から成長障害を示す重度のIGHDで, 異所性後葉や下垂体茎断裂を認めない場合は, *GH-1* または *GHRHR* 変異の検索の適応と考えられる. 自験例で認められたGH反応性の残存や, 下垂体サイズの保持が, 幼少期に限って認められる所見なのか否かの検討が, 今後の課題である.

文　献

1) Lindsay R et al : J Pediatr 125 : 29, 1994.
2) Vimpani GV et al : Br Med J 2 (6084) : 427, 1977.
3) Rona RJ et al : Arch Dis Child 52 : 197, 1977.
4) Cogan JD et al : Adv Pediatr 45 : 337, 1998.
5) Mayo KE et al : Recent Prog Horm Res 55 : 237, 2000.
6) Pombo CM et al : Endocrinology 141 : 2113, 2000.
7) Gaylinn BD : Receptors Channels 8 : 155, 2002.
8) Godfrey P et al : Nat Genet 4 : 227, 1993.
9) Lin SC et al : Nature 364 : 208, 1993.
10) Wajnrajch MP et al : Nat Genet 12 : 88, 1996.
11) Maheshwari HG et al : J Clin Endocrinol Metab 83 : 4065, 1998.
12) Netchine I et al : J Clin Endocrinol Metab 83 : 432, 1998.
13) Wajnrajch MP et al : Am J Med Genet A 120A : 77, 2003.
14) Salvatori R et al : J Clin Endocrinol Metab 84 : 917, 1999.
15) Alatzoglou KS et al : J Clin Endocrinol Metab 94 : 3191, 2009.
16) de Graaff LC et al : Clin Endocrinol (Oxf) 70 : 742, 2009.
17) 堀川玲子：日本臨牀 60 : 297, 2002.
18) Inoue H et al : Clin Endocrinol (Oxf) 74 : 223, 2011.
19) Soneda A et al : Growth Horm IGF Res 23 : 89, 2013.
20) Barreto-Filho JA et al : J Clin Endocrinol Metab 87 : 2018, 2002.

＜臨床報告＞

生後７カ月時に嘔吐と意識障害で発症したグルタル酸血症Ⅱ型の一例

神奈川県立こども医療センター　内分泌代謝科
湊川真理、室谷浩二、花川純子、大戸佑二、朝倉由美、安達昌功

要旨

生後７カ月時に嘔吐と意識障害で発症したグルタル酸血症Ⅱ型の一女児例を報告する。タンデムマス・スクリーニング導入以前の症例である。数日間の上気道炎症状の後、嘔吐、意識障害を呈した。脳症は否定的で、尿中有機酸分析、タンデムマス分析から、グルタル酸血症Ⅱ型と診断した。Immunoblottingにより ETFDH 蛋白の発現を認めなかったことから、*ETFDH* 遺伝子の変異解析を行い、複合ヘテロ接合体変異（Exon 3 に c.251C＞T、p.A84V、および、Exon 8 に c.890G＞T、p.W297L）を同定し、グルタル酸血症Ⅱ型と確定した。診断確定後、リボフラビン内服を継続して、定期的に外来経過観察中である。その後５年半の間に、インフルエンザウイルス感染、ロタウイルス胃腸炎などで計５回、各２～３日間の入院加療を行ったが、軽度の代謝性アシドーシスや低血糖を示すことはあるものの、重篤な急性代謝不全に陥ることはなく、毎回輸液療法のみで改善している。身体発育および精神運動発達は順調で、現在、普通学級に通学中である。初診時の対応、診断が適切になされたことが、予後の改善に役立ったと考えられる。

【はじめに】

グルタル酸血症Ⅱ型は、有機酸代謝異常症の一種で、別名、複合アシル-CoA 脱水素酵素欠損症ともいわれる、ミトコンドリアの電子伝達フラビン蛋白（ETF）あるいは ETF 脱水素酵素の遺伝子変異が原因で起こる常染色体劣性遺伝形式の疾患である。これらの機能不全により ATP 産生が障害されると共に、脂肪酸 β 酸化系酵素など複数の酵素の障害が惹起され、血中に中長鎖を中心としたアシルカルニチンが、また、尿中にエチルマロン酸やグルタル酸などの特徴的な有機酸が増加する[1]。

最重症の患児では、先天性の脳奇形や腎奇形が認められ、新生児早期から心筋障害が急速に進行し、呼吸障害や意識障害などの重篤な状態を呈するため、治療は困難である。一方、軽症～中等症の患児では、乳幼児期にかけて、低血糖症や代謝性アシドーシス、高アンモニア血症により嘔吐、意識障害や痙攣などを繰り返し、脳・筋障害や突然死を来すことがある。これらの患者では、早期治療により脳・筋障害を改善したり、突然死を防いだりすることが出来るため、早期診断が極めて重要である。

今回われわれは、タンデムマス・スクリーニング導入以前に、嘔吐と意識障害で発症したグルタル酸血症Ⅱ型の一乳児例を経験したので報告する。

【症例】７カ月　女児

【主訴】嘔吐・意識障害

【妊娠・分娩歴】妊娠経過に特記すべきことなし。在胎40週０日、頭位経腟分娩、体重 3,122g（+0.8SD）、身長50.0cm（+0.3SD）、頭囲32.5cm（−0.3SD）、Apgar score 9/10で出生。哺乳良好で他に問題なく、日齢５に母と一緒に退院した。新生時ガスリー検査に異常なし（タンデムマス分析は未施行）。

【既往歴】特記すべきことなし

【発達歴】定頸・あやし笑い４ヶ月、寝返り・座位６ヶ月。健診等で異常を指摘されたことはなし。

【家族歴】血族婚なし、遺伝性疾患や新生児死亡などの家族歴なし。５歳姉：気管支喘息

【現病歴】生後７ヶ月時、３日間咳嗽が続いていた。発熱なく活気あり、哺乳も良好であったため自宅で様子をみていた。咳嗽出現４日目に嘔吐が

出現。離乳食や母乳を摂取しようとせず、その後嘔吐を繰り返し、開眼しなくなったため近医に受診した。近医受診時、採血等の痛み刺激に反応なく、開眼せず半昏睡の状態であった。血糖値30台と低血糖あり、肝腫大も認めたため、当院に精査・加療目的で緊急転院となった。来院数日前より、両親と姉が下痢症状を訴えていたが、児に下痢症状は認めなかった。

【入院時現症】全身状態不良。JCS 100-200（採血時に声は出すが開眼せず、四肢の動きはほとんどなし）。体重9,015g（+1.5SD）。脈拍数150回/分、呼吸数40回/分、SpO2 100%（room air）、体温 37.3℃。頭部硬直なし。大泉門平坦。眼瞼結膜：貧血なし、黄疸なし、瞳孔両側2mm、対光反射迅速。咽頭発赤なし。呼吸音：清。心音：整・雑音なし。腹部：平坦・軟、右季肋下に硬い肝臓を3cm触知、脾腫大なし。麻痺はないが体動ほとんどなし。深部腱反射：亢進なし、減弱なし、左右差なし。

【検査所見】（異常値には下線を記す）

＜血算＞　WBC 17400/μL（N 67%、L 30%）、RBC 351×10⁴/μL、Hct 28.3%、Hb 9.8g/dL、Plt 35.8×10⁴/μL

＜生化学＞　Na 139mEq/L、K 4.3mEq/L、Cl 105 mEq/L、Ca 8.7mg/dL、iP 5.5mg/dL、Mg 2.1mg/dL、TP 4.8g/dL、Alb 3.7g/dL、T-Bil 0.6mg/dL、D-Bil 0.2mg/dL、ChE 163IU/L、γGTP 14IU/L、AMY 151IU/L、胆汁酸 13.0μmol/L、AST 342IU/L、ALT 184IU/L、LDH 522IU/L、CK 882IU/L、BUN 14.1 mg/dL、Cr 0.21mg/dL、Glu 60mg/dL、CRP 0.11 mg/dL、NH_3 79μmol/L、NSE 11.9μg/mL、Lac 20.0 mg/dL、ピルビン酸 1.85mg/dL、AcAc 442μmol/L、3-OHBA 801μmol/L、Cu 37μg/dL、セルロプラスミン 9mg/dL、AFP 193.6mg/dL、HBs抗原 陰性、HBs抗体 陰性、HCV抗体 陰性

＜凝固系＞　APTT 40.1sec、PT-INR 1.42、ATⅢ 30%、Fib 55mg/dL、D-dimer 0.54μg/mL

＜血液ガス（静脈）＞　pH 7.367、pCO_2 31.4、HCO_3^- 17.6、BE −6.8mmol/L

＜髄液検査＞　細胞数 2/3（Mono）、蛋白 24.0 mg/ml、糖 71.5mg/dL、NSE 2.5ng/mL、Lac 19.7 mg/dL、ピルビン酸 1.66mg/dL

＜糞便検査＞　ロタウイルス陰性、アデノウイルス陰性

＜血漿アミノ酸検査＞ Aspartic acid 4.9nmol/mL、Hydroxyproline 34.5nmol/mL、Valine 71.9nmol/mL、Cystine 15.9nmol/mL、Isoleucine 16.9nmol/mL（低値）、Leucine 33.3nmol/mL（低値）、Monoethanolamine 26.1nmol/mL（高値）、Arginine 30.2 nmol/mL

＜尿中有機酸分析＞　ジカルボン酸尿、軽度ケトーシス、Ethylmalonate、glutarate、2-OH-glutarateの排泄増加（図1）

＜タンデムマス分析＞　遊離カルニチンの低下、中鎖～長鎖アシルカルニチンの広範な上昇あり（表）

＜脳波検査＞　全般性に徐派の混入あり、発作性異常波なし

＜頭部CT＞　脳浮腫なし、出血病変なし、奇形なし、皮髄境界は月齢相当。

＜頭部MRI＞　（入院翌日に施行）拡散強調像を含め脳実質に異常信号を認めず、皮髄境界は月齢相当。頭蓋内異常を認めず。

＜腹部超音波検査＞　肝実質および両側腎皮質の輝度がびまん性に上昇。肝腫大軽度。Glycogen strage diseaseなどの代謝疾患の可能性が疑われる。

【入院後経過】

入院時、意識障害および嘔吐に加え、脳波検査で全般性徐派を認め、髄液検査で細胞数の増加がなかったことから脳症を疑って、総合診療科で精査・加療を行なった。入院翌日、頭部MRI検査（拡散強調像を含む）で異常を認めず、また、脳波も正常化していた。開眼し時折目が合うようになり意識も入院時に比べて改善傾向であった。髄液のNSE、乳酸、ピルビン酸等の上昇なく、脳症は否定的となった。代謝性アシドーシス、ケトン体の上昇、肝酵素上昇、凝固異常を認めたことから、何らかの要因による肝機能障害や代謝性疾患を疑い、アシドーシスの補正等行いながら、内分泌代謝科にコンサルトとなった。

当院来院時、低血糖および代償性の代謝性アシドーシスに加えて、凝固障害を合併し、意識レベルは傾眠傾向で、ほとんど開眼しなかった。入院後から低血糖に対してGIR 5.4mg/kg/minで輸液を開始したが、血糖値が安定せず、血糖値をモニター