Endocrine Journal 2000, **47** (6), 777–782

NOTE

Japanese Familial Patients with Male-Limited Precocious Puberty

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Abstract. Familial male-limited precocious puberty (FMPP) is a rare disease caused by constitutively activating mutations in the luteinizing hormone receptor (LH-R) gene. In the present study, we analyzed the LH-R gene in members of a Japanese FMPP family. Two males of the family were affected and had a heterozygous M398T mutation; one patient developed pubertal signs as early as 2 years of age, and the other at 6 years of age. Both patients had elevated serum testosterone levels and prepubertal gonadotropin secretions. The father of the latter patient carried the M398T mutation, but lacked history of precocious puberty. Thus, phenotypic differences were observed in the three males with the same LH-R mutation belonging to the same family. In summary, we have described a Japanese family with FMPP.

Key words: Familial male-limited precocious puberty (FMPP), Luteinizing hormone receptor (LH-R), Constitutively activating mutation, Phenotypic heterogeneity

(*Endocrine Journal* **47**: 777–782, 2000)

FAMILIAL male-limited precocious puberty (FMPP) is a form of precocious puberty that is limited to males, and the puberty is gonadotropin-independent characterized by low levels of serum luteinizing hormone (LH) and prepubertal LH and follicular-stimulating hormone (FSH) responses to gonadotropin-releasing hormone (GnRH) [1]. Recent studies demonstrated that FMPP is caused by mutations in the LH receptor (LH-R) gene which result in the constitutive activation of the LH-R [2, 3]. In Japan, four "sporadic" cases with male-limited precocious puberty have been reported [4] and the genetic analyses revealed that they are heterozygous for activating mutations in the LH-R gene [5–7].

The present study is the report of a first Japanese family with FMPP and describes the phenotypic heterogeneity of the FMPP family.

Received: April 6, 2000
Accepted: August 7, 2000
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Case Report

The family tree is given in Fig. 1. The proband (Patient 1) was the first child of healthy unrelated Japanese parents. He was born in 1974 by vaginal aspiration delivery at 39 weeks and 6 days of gestation after uncomplicated pregnancy. His birth weight was 3730 g and length 51.5 cm. His growth chart is given in Fig 2A. By the age of 2 years it was noticed that he had accelerated increase in height, rapid penile enlargement and appearance of pubic hair. When first seen at 2 years and 4 months, his height was 93.3 cm (+1.7 SD) and his weight was 15.0 kg; Tanner stages were G3 and PH2. Testicular sizes were 32 × 21 mm on the right and 22 × 15 mm on

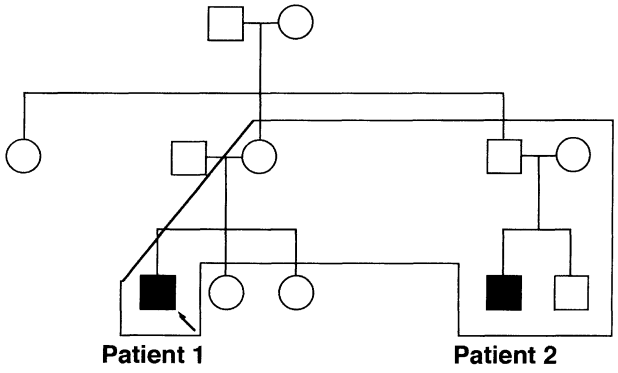

Fig. 1. Family tree. The arrow indicates an index case. Family members included in the genetic analysis are surrounded by the lines.

the left, penile length was 73 mm. Bone age was 3 years. Serum testosterone was 300 ng/dl (normal range for prepubertal boys: <10 ng/dl). Serum LH and FSH levels were <1.0 (normal range for prepubertal boys: 1.1–7.5) mIU/ml and 1.15 (normal range for prepubertal boys: <9.7) mIU/ml, respectively. After administration of GnRH, they increased to 9.8 (normal range for prepubertal boys: 6.8–22.4) mIU/ml and 7.4 (normal range for prepubertal boys: 6.9–26.5) mIU/ml, respectively. He was treated with medroxyprogesterone acetate and/or cyproterone acetate until 12 years and 3 months, when he was 156.3 cm tall (+0.9 SD). He is 160.5 cm tall (−1.8 SD) and at full puberty. He has two younger sisters. His parents and sisters presented normal pubertal development.

Patient 2 is a maternal cousin of Patient 1. He was the first child of healthy unrelated Japanese parents. He was born in 1985 by vaginal natural delivery after uncomplicated 39-week gestation. His birth weight was 3060 g and length 48.0 cm. His growth chart is given in Fig 2B. At 3 years of age he was pointed out to have undescended testes, and was referred to the Department of Urology in our

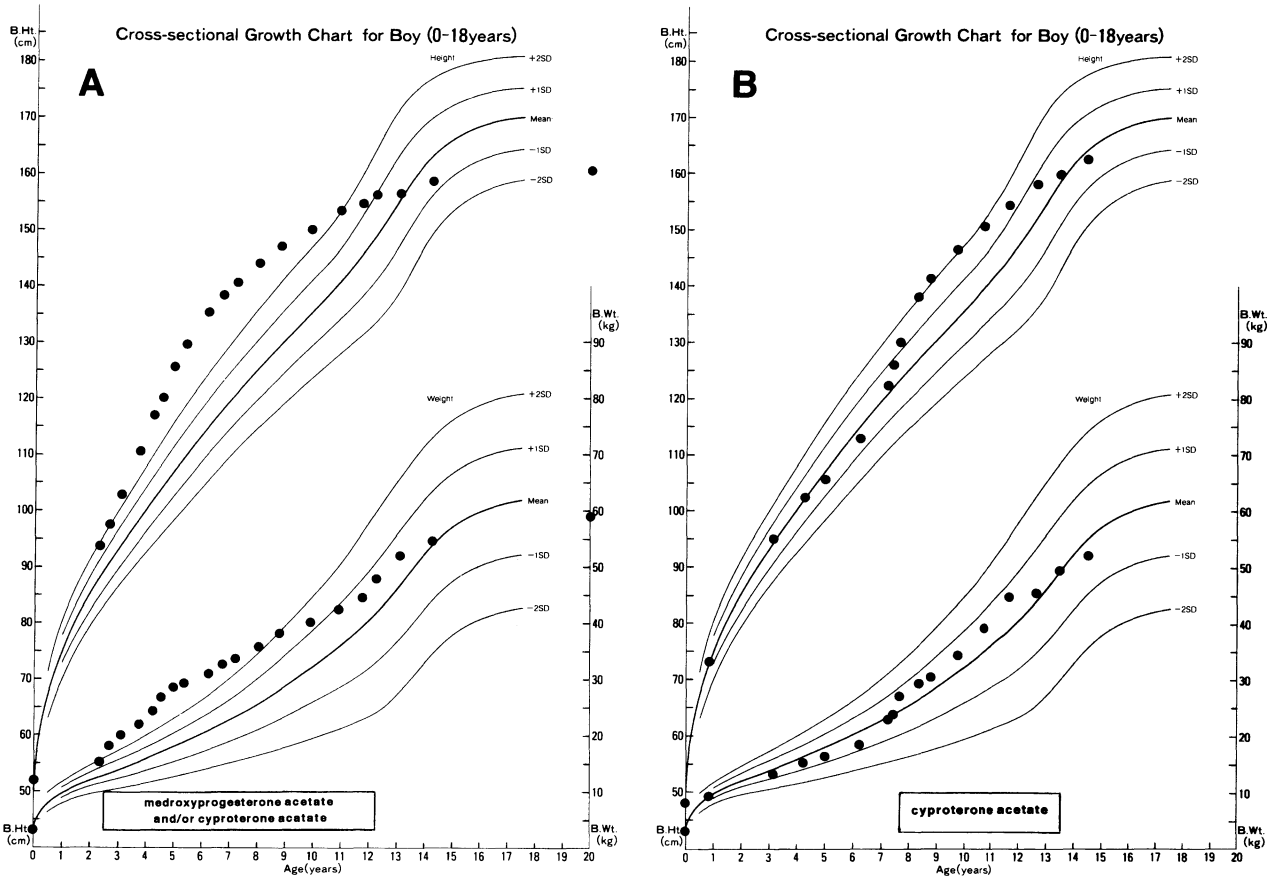

Fig. 2. Longitudinal growth record of the FMPP patients. A, Growth chart of Patient 1. B, Growth chart of Patient 2.

hospital. He was diagnosed as having migratory testes, and has been followed up since then. Because he started to grow rapidly and present second sexual characteristics at the age of 6 years, he was referred to us. When first seen at 7 years and 5 months of age, his height was 126.0 cm (+0.8 SD) and his weight was 24.0 kg; Tanner stages were G3 and PH2. Testes have descended into the scrotum. Testicular volumes were 6 ml on the right and 5 ml on the left, penile length was 64 mm. Bone age was 8 years and 6 months. Serum testosterone was 379 ng/dl. Serum LH and FSH levels were 0.03 (normal range for prepubertal boys: 0.02–0.15) mIU/ml and 0.1 (normal range for prepubertal boys: 0.38–1.11) mIU/ml, respectively. After the administration of GnRH, they increased to 0.65 (normal range for prepubertal boys: 1.70–3.77) mIU/ml and 1.2 (normal range for prepubertal boys: 4.38–9.48) mIU/ml, respectively. He was treated with cyproterone acetate until 13 years and 6 months, when he was 160.0 cm tall (+0.2 SD). He has one younger brother, who was born in 1988 and is still prepubertal at the age of 11 years. His father is a younger sibling of the mother of Patient 1 (Fig. 1) and 166.5 cm tall (−0.8 SD). The father as well as the mother of Patient 2 is said to have entered puberty normally.

Bone age estimation

Bone age was measured by the RUS method using the Japanese specific bone age standard on TW2 [8].

Hormone measurements

Serum LH and FSH in Patient 1 were measured by the double antibody radioimmunoassay (RIA) method using kits from Daiichi Radioisotope Institute, Tokyo, Japan. Serum LH and FSH in Patient 2 were determined by the immunofluorometric assay method using Delfia kits from Pharmacia Biosystems, Tokyo, Japan. Serum testosterone was measured by specific RIA.

Materials and Methods

DNA amplification and sequence analysis of the LH receptor gene

Family members included in the genetic analysis are indicated in Figure 1. Genomic DNA was isolated from peripheral blood of the family members by proteinase K digestion and phenol/chloroform extraction. Exon 11 of the LH receptor gene was amplified in three overlapping fragments. The primer sequences are listed in Table 1. Polymerase chain reactions (PCR) were performed in a 100-μl mixture containing 0.2 μg genomic DNA, 0.001% gelatin, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 100 μM of each dNTP, and 250 nM of the sense primer and the antisense primer, and 2.5 U *Taq* DNA polymerase (Takara Shuzo Co., Ltd., Otsu, Japan). After an initial step of denaturation at 95°C for 4 min, 30 cycles of denaturation at 95°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 0.5 min were followed by an additional extension at 72°C for 3 min. The amplified PCR products were fractionated on a 1% agarose gel (Bio-Rad Laboratories, Richmond, CA, USA), and electroeluted from the gel and purified [9]. The purified PCR products were directly sequenced using [α-^{35}S]-dATP (NEN Life Science Products, Inc., Boston, MA, USA) and a Sequenase kit (Amersham Japan Ltd., Tokyo, Japan) with the

Table 1. Primer sequences

Primer	Direction	Sequence	Location
LH-R S1	Sense	5′-TTATTCTTCCATGCTTGCTGAGAGT-3′	1038–1062
LH-R AS1	Antisense	5′-TAATCGCAGCTTTTGGTCCAGGTGA-3′	1440–1416
LH-R S2	Sense	5′-CACTGCTGGCTTTTTCACTGTATT-3′	1320–1343
LH-R AS2	Antisense	5′-TGAAGGCAGCTGAGATGGCAAAAA-3′	1783–1760
LH-R S3	Sense	5′-ATGGCAATCCTCATCTTCACCGATT-3′	1711–1735
LH-R AS3	Antisense	5′-TAGAGGTCTCTTGCCTAATGTACCT-3′	2235–2211

same primers as those used for PCR.

Restriction analysis

The mutation found in the patients turned out to create a new restriction site for *Pml* I. Therefore, we confirmed the mutation in the patients and their family members by digestion of the PCR products with the restriction enzyme *Pml* I (New England Biolabs Inc., Beverly, MA, USA) and subsequent separation on a 1% Nusieve GTG (FMC BioProducts, Rockland, ME, USA)/1% agarose gel.

Informed consent for the genetic analysis was obtained from each subject according to the institutional guidelines of the National Children's Medical Research Center.

Results

The PCR products amplified by each set of the primer pairs were of expected sizes. The direct sequencing of the PCR products from the patients revealed a heterozygous missense mutation changing codon 398 (ATG) encoding Met to ACG encoding Thr (M398T) (Fig. 3A).

Since the M398T mutation creates a new recognition site for a restriction enzyme *Pml* I (CACGTG), the PCR products from the family members were digested with the enzyme and analyzed on a 1% Nusieve GTG/1% agarose gel. As shown in Fig. 3B, the patients, the mother of Patient 1, and the father of Patient 2 were heterozygous for the M398T mutation. The mother and the brother of Patient 2 were homozygous for the wild-type allele.

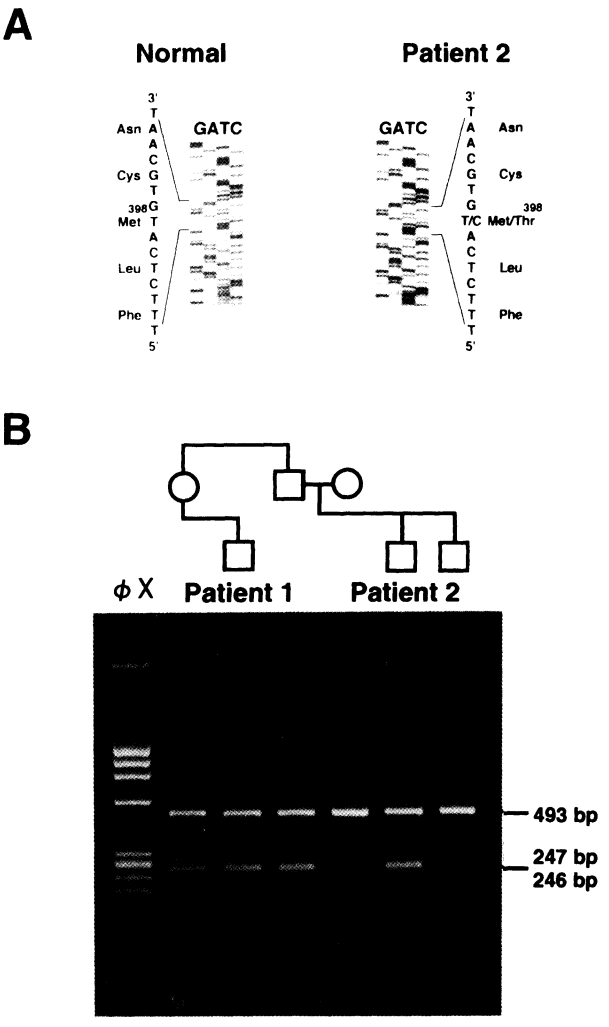

Fig. 3. Genetic analysis of the LH-R gene. A, Direct sequencing of the LH-R gene. Patient 2 is heterozygous for a T to C transition at nucleotide 1193. This mutation results in substitution of Thr for Met at amino acid residue 398 (M398T mutation). B, *Pml* I restriction enzyme analysis of PCR products. Only DNA containing the M398T mutation is digested by *Pml* I. DNAs from the patients, the mother of Patient 1 and the father of Patient 2 yielded undigested (493-bp) and digested (246- and 247-bp) fragments, indicating they are heterozygous for the mutation. DNAs from the mother and the brother of Patient 2 remained undigested, indicating they are homozygous for the wild-type allele. ϕ X indicates *Hae* III-digested ϕ X174 RF DNA, which was used as size markers.

Discussion

The functional consequences of the M398T mutant LH-R were previously studied by Kraaji *et al.* [10] and Yano *et al.* [7]. Both studies demonstrated that the expression of the M398T mutant LH-R causes markedly increased cyclic AMP production in the absence of the ligand. Therefore, the M398T mutation is considered to be the cause of FMPP in our patients.

Yano and his colleagues analyzed the LH-R gene in four Japanese patients with "sporadic" male-limited precocious puberty and demonstrated that two of them are actually hereditary cases without family history [5–7]. Therefore, it was clearly demonstrated

that there exist hereditary patients with male-limited precocious puberty in Japan, but familial occurrence of male-limited precocious puberty in Japan has not been described. In the present study, we reported the familial occurrence of male-limited precocious puberty for the first time in Japan.

It is interesting to note that three males with the M398T mutation in our family presented quite different phenotypes; Patient 1 developed signs of precocious puberty as early as 2 years of age, Patient 2 at 6 years of age, whereas the father of Patient 2 lacked history of precocious puberty. Similar differences in phenotype were observed in the FMPP families caused by the M398T mutation [7, 17]. The phenotypic differences seem to be unique to the M398T mutation, since no phenotypic heterogeneity was reported in FMPP families caused by the other mutations in the LH-R gene [2, 3, 11–16]. The reason for the phenotypic differences cannot be currently explained, but other genetic or environmental factors must have affected the clinical presentation.

In summary, we have described a Japanese family with FMPP caused by the M398T mutation in the LH-R gene and demonstrated that there exist phenotypic differences in the family.

Acknowledgments

The authors wish to thank Ms. Shoko Mikami and Ms. Atsuko Nagashima-Miyokawa for technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Science and Culture, Japan, Grants for Pediatric Research (10C-2 and 10C-3) from the Ministry of Health and Welfare, Japan, and a Grant for Liberal Harmonious Research Promotion System from the Science and Technology Agency, Japan.

References

1. Holland FJ (1991) Gonadotropin-independent precocious puberty. *Endocrinol Metab Clin North Am* 20: 191–210.
2. Shenker A, Laue L, Kosugi S, Merendino Jr JJ, Minegishi T, Cutler Jr GB (1993) A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. *Nature* 365: 652–654.
3. Kremer H, Mariman E, Otten BJ, Moll Jr GW, Stoelinga GBA, Wit JM, Jansen M, Drop SL, Faas B, Ropers HH, Brunner HG (1993) Cosegregation of missense mutations in the luteinizing hormone receptor gene with familial male-limited precocious puberty. *Hum Mol Genet* 2: 1779–1783.
4. Ito Y, Yano K, Mitamura R, Oka R, Okuno A, Kataoka N, Hiraba K, Moriya N (1992) Sporadic testotoxicosis in Japanese children: report of 4 cases. *Clin Pediatr Endocrinol* 1: 95–100.
5. Yano K, Hidaka A, Saji M, Polymeropoulos MH, Okuno A, Kohn LD, Cutler Jr GB (1994) A sporadic case of male-limited precocious puberty has the same constitutively activating point mutation in luteinizing hormone/choriogonadotropin receptor gene as familial cases. *J Clin Endocrinol Metab* 79: 1818–1823.
6. Yano L, Saji M, Hidaka A, Moriya N, Okuno A, Kohn LD, Cutler Jr GB (1995) A new constitutively activating mutation in the luteinizing hormone/choriogonadotropin receptor gene in cases of male-limited precocious puberty. *J Clin Endocrinol Metab* 80: 1162–1168.
7. Yano L, Kohn LD, Saji M, Kataoka N, Okuno A, Cutler Jr GB (1996) A case of male-limited precocious puberty caused by a point mutation in the second transmembrane domain of the luteinizing hormone choriogonadotropin receptor gene. *Biochem Biophys Res Commun* 220: 1036–1042.
8. Murata M (1993) Japanese specific bone age standard on the TW2. *Clin Pediatr Endocrinol* 2 (suppl 3): 35–41.
9. Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning—A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, 166–167.
10. Kraaji R, Post M, Kremer H, Milgrom E, Epping W, Brunner HG, Grootegoed JA, Themmen PN (1995) A missense mutation in the second transmembrane segment of the luteinizing hormone receptor causes familial male-limited precocious puberty. *J Clin Endocrinol Metab* 80: 3168–3172.
11. Kosugi S, Dop CV, Geffner ME, Rabl W, Carel JC, Chaussain JL, Mori T, Merendino Jr JJ, Shenker A (1995) Characterization of heterozygous mutations causing constitutive activation of the luteinizing hormone receptor in familial male precocious puberty. *Hum Mol Genet* 3: 183–188.
12. Laue L, Chan WY, Hsueh AJW, Kudo M, Hsu SY, Wu SM, Blomberg LA, Cutler Jr GB (1995) Genetic

heterogeneity of constitutively activating mutations of the human luteinizing hormone receptor in familial male-limited precocious puberty. *Proc Natl Acad Sci* 92: 1906–1910.

13. Laue L, Wu SM, Kudo M, Hsueh AJW, Cutler Jr GB, Jelly DH, Diamond FB, Chan WY (1996) Heterogeneity of activating mutations of the human luteinizing hormone receptor in male-limited precocious puberty. *Biochem Mol Med* 58: 192–198.
14. Kawate N, Kletter GB, Wilson BE, Netzloff ML, Menon KM (1995) Identification of constitutively activating mutation of the luteinizing hormone receptor in a family with male limited gonadotrophin independent precocious puberty (testotoxicosis). *J Med Genet* 32: 553–554.
15. Cocco S, Meloni A, Marini MG, Cao A, Moi P (1996) A missense (T577I) mutation in the luteinizing hormone receptor gene associated with familial male-limited precocious puberty. *Hum Mutation* 7: 164–166.
16. Kremer H, Martens JWM, van Reen M, Verhoef-Post M, Wit JM, Otten BJ, Drop SLA, Delemarre-van de Waal HA, Pombo-Arias M, De Luca F, Potau N, Buckler JMH, Jansen M, Parks JS, Latif HA, Moll GW, Epping W, Saggese G, Mariman ECM, Themmen APN, Brunner HG (1999) A limited repertoire of mutations of the luteinizing hormone (LH) receptor gene in familial and sporadic patients with male LH-independent precocious puberty. *J Clin Endocrinol Metab* 84: 1136–1140.
17. Evans BAJ, Bowen DJ, Smith PJ Clayton PE, Gregory JW (1996) A new point mutation in the luteinising hormone receptor gene in familial and sporadic male limited precocious puberty: genotype does not always correlate with phenotype. *J Med Genet* 33: 143–147.