CLINICAL STUDY

A novel mutation of the *KAL1* gene in monozygotic twins with Kallmann syndrome

Takeshi Matsuo¹, Shingo Okamoto¹, Yukiko Izumi², Akiko Hosokawa³, Takashi Takegawa¹, Hiroshi Fukui¹, Zaw Tun⁴, Katsuya Honda⁴, Ryoji Matoba⁴, Ke-ita Tatsumi² and Nobuyuki Amino²

¹Third Department of Internal Medicine, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan, ²Department of Laboratory Medicine, Osaka University Graduate School of Medicine D2, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan, ³Hikari Clinic, 1-8-8 Gakuen-kita, Nara 631-0036, Japan and ⁴Department of Legal Medicine, Osaka University Graduate School of Medicine F3, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

(Correspondence should be addressed to Y Izumi; Email: yizumi@labo.med.osaka-u.ac.jp)

Abstract

Objective: Kallmann syndrome is defined by the association of hypogonadotropic hypogonadism and anosmia. The *KAL1* gene is responsible for the X-linked form of Kallmann syndrome. In this study we describe monozygotic twins with Kallmann syndrome due to the same mutation in the *KAL1* gene.

Design: We studied male monozygotic twins with Kallmann syndrome.

Methods: We analyzed the *KAL1* gene using the PCR-direct sequencing method. The twins' mother was examined for the identified mutation.

Results: We identified a 14 bp deletion from codon 419 in exon 9 (Pro419del14) in both *KAL1* genes of the twins. This was a novel mutation in the *KAL1* gene and was responsible for Kallmann syndrome. As Pro419del14 was not detected in the mother of the twins, Pro419del14 was a germline mutation originating from them. These monozygotic twins showed different LH and FSH responses to LH–RH stimulation and different phenotypes such as complications, physiques and psychiatric characters.

Conclusions: We report an identical *KAL1* gene mutation in the monozygotic twins with Kallmann syndrome. As these monozygotic twins showed different phenotypes in some respects, we suggest that factors other than mutations in the *KAL1* gene affect the symptomatic features of Kallmann syndrome.

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Introduction

Kallmann syndrome is defined by the association of hypogonadotropic hypogonadism and anosmia. Familial cases are transmitted as X-linked, autosomal recessive or autosomal dominant traits, but sporadic cases have also been reported. The *KAL1* gene, a candidate gene for X-linked Kallmann syndrome, was cloned in 1991 by Ballabio *et al.* (1) and Legouis *et al.* (2). This gene consists of 14 exons spanning approximately 210 kb on Xp22.3 (3, 4). The *KAL1* gene probably encodes a protein functioning in neuronal migration and axonal targeting (1, 2). Numerous *KAL1* gene mutations responsible for Kallmann syndrome have been reported in X-linked families and sporadic cases (5–16). We describe here the first reported case of monozygotic twins with Kallmann syndrome, and show that they had a novel *KAL1* gene abnormality.

Case report

Two 19-year-old men (Cases 1 and 2) were admitted to our hospital for delay of puberty. They were twins. At birth Case 1 was 49.5 cm long and weighed 3100 g, while Case 2 was 44.0 cm long and weighed 1990 g. Case 2 was diagnosed as having a mild ventricular septal defect. Both grew normally but Case 1 was always taller and heavier than Case 2 (Fig. 1). Their parents had noticed since their infancy that they could not recognize any smells. Even at 16 years old, they had no pubertal development. On physical examination, Case 1 was obese; he was 181 cm tall and weighed 100 kg. His penis was 4.8 cm in length, and his testes were both 2 ml in volume. Smell tests disclosed severe hyposmia. He also had exotropia. Case 2 was 173 cm tall and weighed 76 kg. His penis was 4.2 cm in length, and his testes were both 1 ml in volume. Smell tests disclosed severe

Figure 1 Growth curves. Case 1 was always taller and heavier than Case 2.

hyposmia, as for Case 1. Congenital hyposmia and hypogonadism were not found among their relatives.

The basal FSH, LH and testosterone levels of Cases 1 and 2 were all below adult reference values, as shown

in Table 1. Their serial LH–RH provocation tests showed stepwise LH and FSH elevations. On the seventh day, both LH and FSH responded normally and the peak values for Case 2 were higher than those

Figure 2 Serial LH-RH provocation tests (200 µg/day i.m. for 7 days). On the seventh day, both LH and FSH responded normally, and the peak values for Case 2 were higher than those for Case 1.

for Case 1 (Fig. 2). Clomiphene citrate provocation tests showed neither LH nor FSH response in either case. Evaluation of the other pituitary hormones showed normal basal levels (Table 1). These findings were consistent with hypogonadotropic hypogonadism with hyposmia, and these twins were diagnosed with Kallmann syndrome. Abdominal ultrasound examination showed no renal abnormality in either case.

As eight kinds of human leukocyte antigen (HLA) type analysis showed A24(9), A26(10), B7, B60(40), Cw7, Cw3, DR1 and DR11(5) in both cases and the twins had a common placenta at birth, they were considered to be monozygotic twins. Confirmation of twin monozygosity was obtained by analysis of DNA polymorphisms. The zygosity analysis gave a probability of monozygosity of 99.999%. Psychologically, the

Yatabe–Guilford personality test (17) showed that Case 1 was an extrovert and Case 2 was an introvert.

Methods

Endocrinological studies

Serum LH, FSH, GH, somatomedin C (Sm.C), TSH and estradiol (E₂) were measured by solid phase immunoradiometric assays using the SPAC-S LH, SPAC-S FSH (Daiichi RI, Tokyo, Japan), GH 'Daiichi' (Daiichi RI, Tokyo, Japan), somatomedin C-II 'Ciba Corning' (Ciba Corning Diagnostic, Tokyo, Japan), TSH-RIABEAD II (Dainabot, Tokyo, Japan) and estradiol ¹²⁵I (CIS Diagnostic, Tokyo, Japan) kits, respectively. Testosterone, tri-iodothyronine (T₃) and thyroxine (T₄) were measured by radioimmunoassays using the DPC total testosterone (DPC, Tokyo, Japan), T-3 RIABEAD II (Dainabot) and T-4 RIAKIT II (Dainabot) kits, respectively.

Table 1 Basal levels of pituitary and sex hormones in Cases 1 and 2. Values in parentheses indicate reference range and units.

	Case 1	Case 2
GH (<0.5 ng/ml)	0.1	0.2
Sm.C (259–351 ng/ml)	442	415
TSH (0.24–3.7 µU/ml)	2.0	1.4
T ₃ (0.7–2.1 ng/ml)	1.2	1.4
T ₄ (4.5–12.3 µg/dl)	7.4	8.1
LH (1.8–5.2 mIU/ml)	<0.5	<0.5
FSH (2.9–8.2 mIU/ml)	<0.5	<0.5
Testosterone (2.7–10.7 ng/ml)	0.4	0.3
E ₂ (15–60 pg/ml)	21	20

Serial LH–RH provocation tests The serum LH and FSH responses to LH–RH (200 µg/day i.m. for 7 days) were determined every 30 min for 2 h on the first and the seventh days.

Clomiphene citrate provocation tests Clomiphene citrate (100 mg/day for 7 days) was administered, and serum LH and FSH were measured before and after stimulation.

Figure 3 Sequence analysis of exon 9 in the *KAL1* gene. The mutation in the twins was a 14 bp deletion starting at codon 419 in exon 9. This mutation was not detected in their mother.

DNA extraction, PCR and sequencing

Genomic DNA was extracted from peripheral leukocytes using a simple salting out method (18). All 14 exons of the *KAL1* gene were amplified by PCR as previously reported (16) using primers described by Hardelin *et al.* (8). The PCR-amplified fragments were purified and submitted to a sequencing reaction using the ABI PRISM dye terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase, FS (Perkin-Elmer, Applied Biosystems Division, Foster City, CA, USA). For all 14 exons, both DNA strands were sequenced using an ABI PRISM 310 genetic analyzer (Perkin-Elmer).

HLA type analysis

HLA-A, -B, -C and -DR loci were analyzed using the Terasaki-NIH standard method.

Analysis of DNA polymorphisms

DNA polymorphisms were analyzed by the use of two variable numbers of tandem repeats (ApoB, MCT118) and seven short tandem repeats (TPOX, vWA31, TH01, FES/FPS, HPRTB, ARA, STR X1).

Results

In both *KAL1* genes of the twins the same novel mutation was found, namely, a 14 bp deletion starting at codon 419 in exon 9 (Pro419del14) (Fig. 3). Pro419del14 resulted in frameshift and truncation of the *KAL1* protein due to a premature stop codon at codon 440. Pro419del14 was not detected in the *KAL1* gene on both X chromosomes of the twins' mother.

Discussion

Here we reported monozygotic twins with Kallmann syndrome due to a 14 bp deletion in exon 9 starting at codon 419 in the *KAL1* gene. As Pro419del14 results in frameshift and truncation of the *KAL1* protein due to a premature stop codon at codon 440, the third and fourth fibronectin type III-like repeats of the *KAL1* protein, a domain involved in the processes of neuronal migration and axonal targeting, are lost (1, 2) and so this mutation is considered to be responsible for Kallmann syndrome.

This is the first report on monozygotic twins with an identical *KAL1* gene mutation. Interestingly, these monozygotic twins, possessing the same genotype, showed different phenotypes in some respects. First, although the serial LH–RH provocation tests showed stepwise LH and FSH elevations, the response in Case 2 was higher than that in Case 1. Secondly, Case 1 had exotropia, while Case 2 had a ventricular septal defect. Although Kallmann syndrome patients with congenital heart diseases were reported previously, every patient lacked family history and their *KAL1* genes were not analyzed (19). Exotropia had never been reported as complications of Kallmann syndrome with *KAL1* gene mutations. Therefore these abnormalities are thought to be caused by factors other than Pro419del14. Thirdly, Case 1 was taller and heavier than Case 2. Finally, the Yatabe–Guilford personality test showed that Case 1 was an extrovert and Case 2 was an introvert. The presence of these symptomatic heterogeneities in Kallmann syndrome with the same mutation in the *KAL1* gene suggests the existence of other factors that modulate the function of the *KAL1* gene, except for the other genes. Some previous studies reported identical twins with Kallmann syndrome with

discordant phenotypes, but their *KAL1* genes were not analyzed (20, 21). Further, in another family with X-linked Kallmann syndrome with identical partial deletion in the *KAL1* gene, intrafamilial variability was reported. In that family, one of three affected brothers had mild hyposmia and showed normal pubertal progression (11).

Since Pro419del14 was not detected in the twins' mother, we considered that Pro419del14 is a germline mutation originating from the twins. Because all mutations we have found occurred in either probands or the mother (16), and only one large pedigree with the *KAL1* gene mutation has been reported (12), we suggest that mutation in the *KAL1* gene may only rarely be inherited because of the associated male infertility.

In conclusion, here we have reported monozygotic twins with Kallmann syndrome due to a novel 14 bp deletion in exon 9 starting at codon 419 in the *KAL1* gene. Different phenotypes in the twins suggest that other factors might also be important for development of clinical characteristics.

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