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A novel interstitial deletion of *KAL1* in a Japanese family with Kallmann syndrome

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Abstract We identified a novel interstitial deletion that spanned from exons 5 to 10 of *KAL1* in two Japanese brothers with X-linked Kallmann syndrome (KS; MIM no. 308700). Both brothers had hypogonadism, unilateral renal agenesis, and disturbance of the sense of smell, but they had no other neurological manifestations, including mental disturbance. Their mother was confirmed to be an asymptomatic carrier, by use of a comparative multiplex polymerase chain reaction (PCR) analysis. The present patients are further examples of patients with KS without mental disturbance caused by a mutation confined to *KAL1*.

Key words Kallmann syndrome · *KAL1* · Mutation · Anosmia · Hypogonadism

Introduction

Kallmann syndrome (KS; MIM no. 308700) is characterized by hypogonadotropic hypogonadism and disturbance in the sense of smell (hyposmia/anosmia); KS is also often associated with various other symptoms, including mental disturbance. A segregation analysis revealed genetic het-

erogeneity, with X-linked, autosomal recessive, and autosomal dominant modes of transmission (Georgopoulos et al. 1997). The *KAL1* gene (*KAL1*: Genbank accession number, M97252), responsible for the X-linked form of KS, was identified on Xp22.3. Here, we describe a Japanese family with KS associated with a novel interstitial deletion in *KAL1*.

Subjects and methods

Genomic DNAs were prepared from the peripheral blood of the family members and Japanese volunteers by a phenol/chloroform method. Each of the 14 exons of *KAL1* was amplified using polymerase chain reaction (PCR) primers designed according to Hardelin et al. (1992).

The quantity of *KAL1* PCR products was evaluated using *NPCI* PCR products as an internal standard. *NPCI* (Genbank accession number, AF002020) is a gene responsible for Niemann-Pick disease type C (MIM, 257220) and is located on 18q. The PCR contained 40 pmol of each primer (exon 7 of *KAL1* and exon 20 of *NPCI*: 5'-GTA ATG CCC CTC ACT GTC AG-3' and 5'-GTC TTA GCC CAG TCC TCT CC-3'), 2.5 nM of dNTPs (Gene Amp dNTPs mix), 100 ng of genomic DNA, standard PCR buffer, and 1 U of Ampli Taq Gold (Perkin Elmer, Norwalk, CT, USA) in a final volume of 10 µl. Cycling conditions were 10 min at 95°C initial denaturation, 20–35 cycles of 30 s at 95°C, 30 s at 55°C, and 60 s at 72°C, followed by a final extension for 5 min at 72°C. The amplified products were analyzed by electrophoresis on a 6% polyacrylamide gel and with SYBR Green staining (Molecular Probe, Eugene, OR, USA). The intensity of each band was measured using a densitometer (Atto, Tokyo, Japan) and the intensities of *KAL1* bands were evaluated as relative to the intensities of *NPCI* bands (*KAL1/NPCI* ratio). To verify that the obtained ratio reflected the gene dosage in the templates, samples from 10 male and 11 female subjects were also examined.

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Results and discussion

The probands were the two male offspring (patient 1, aged 33 years, and patient 2, aged 29 years) of three offspring (the other was a healthy female) of healthy parents. Both brothers had been operated on for cryptorchism and had received gonadotropin replacement therapy since their early teens. Patient 1 had a standard stature with a height of 174cm (+0.643 SD) and weighted 62kg (body mass index [BMI], 20.5); patient 2 had a height of 177cm (+1.88 SD), weighed 104kg (BMI, 33.2), and was obviously obese (Fig. 1). Both patients had a mild eunuchoid appearance and both had unilateral renal aplasia, as revealed by ultrasonography. Both had normal intelligence and neither had any neurological signs. Although neither of the patients had subjective complaints of hyposmia, both were revealed to have a cryptic disturbance of the olfactory sense, by the use of an alinamin infusion test and the T&T olfactometer test. Both of them had low levels of serum testosterone and luteinizing hormone, follicle stimulating hormone (LH, FSH), which did not respond to human chorionic gonadotropin (hCG) and gonadotropin releasing hormone (GnRH) stimulation, respectively.

All of the *KAL1* exons were successfully amplified from control genomic DNAs, whereas six exons (exons 5–10) were not amplified from the DNAs of the probands (Fig. 2a), suggesting the presence of an interstitial deletion that spanned from exon 5 to exon 10 in both patients' DNAs. To

confirm the carrier status of their mother, we quantified the gene dosage of *KAL1* exon 7 by comparative multiplex (CM)-PCR. This analysis of control male and female DNAs showed that the obtained gene dosage index (*KAL1/NPC1* ratio) reflected the expected gene dosage (0.5 for males and 1.0 for females) within a cycle number of up to 23 (Fig. 2b). Because of deviation of the index from the actual gene dose with cycle numbers more than 23, the cycle was set to 23 in the following experiments. CM-PCR analyses of the mother's DNA under this condition gave a *KAL1/NPC1* ratio of 0.45 (mean; $n = 5$; maximum ratio, 0.49; minimum ratio, 0.40), clearly indicating that she had only one allele of *KAL1* exon 7. As expected, the ratio of the probands' DNAs was zero (Fig. 2c).

Fig. 1. Eunuchoidism of external genitalia (patients 1 and 2) and obesity (patient 2)

Fig. 2a–c. Molecular diagnosis for *KAL1*. **a** Polymerase chain reaction (PCR) products of *KAL1* exons for each individual. The number of each exon is indicated above each lane. Underlines indicate the deleted exons. m, Φ X174/*Hae*III digest. **b** *KAL1/NPC1* ratio (means $\pm$ maximum and minimum) by use of comparative multiplex (CM)-PCR at various cycle numbers. Templates were genomic DNAs from ten male subjects (diamonds) and 11 female subjects (squares). **c** Representative results of five independent determinations by CM-PCR. Values for the *KAL1/NPC1* ratio are given below each lane. m, Φ X174/*Hae*III digest

Table 1. *KAL1* mutations in KS patients

Authors	Year	Location of mutation	Mutation type	Nucleotide change	Predicted amino acid change	Neurological findings
1 Bick et al.	1992	Exon 12–14	Exonic deletion			None
2 Hardelin et al.	1992	Exon 5	Nonsense mutation	nt 711G > A	Y237X	Synkinesia, epilepsy
3		Exon 6	Nucleotide deletion	nt 831 C del	Frameshift	Pes cavus deformity
4		Exon 6	Nonsense mutation	nt 769C > T	R257X	Synkinesia, pes cavus deformity
5		Exon 6	Nonsense mutation	nt 774G > A	Y258X	Synkinesia
6 Meindl et al.	1993	All exons	Contiguous gene syndrome			Mental disturbance
7 Hardelin et al.	1993a	All exons	Exonic deletion			Deafness
8		All exons	Exonic deletion			Synkinesia
9 Hardelin et al.	1993b	Exon 6	Missense mutation	nt 801T > A	N267K	Not mentioned
10		Exon 7	Nucleotide insertion	nt 1016 A ins	Frameshift	Not mentioned
11		Exon 9	Nonsense mutation	nt 1261C > T	Q421X	Not mentioned
12		Exon 9	Nonsense mutation	nt 1267C > T	R423X	Synkinesia
13		Intron 12	Nucleotide change	IVS 12-1G > A	Splicing error	Not mentioned
14 Bouloux et al.	1993		Contiguous gene syndrome			Mental disturbance
15 Klink et al.	1994	?	Contiguous gene syndrome			Mental disturbance
16 Martul et al.	1995	All exons	Complete deletion			None
17 Parenti et al.	1995	Exon 2–14	Exonic deletion		Not expressed	Not mentioned
18 Quinton et al.	1996	All exons	Contiguous gene syndrome			Not mentioned
19		All exons	Exonic deletion			Not mentioned
20		Exon 1	Exonic deletion		Not expressed	Not mentioned
21		Exon 11	Exonic deletion		Frameshift	Not mentioned
22		Exon 12	Nucleotide deletion	nt 1697 C del	Frameshift	Deafness
23 Georgopoulos et al.	1997	Exon 10	Nucleotide deletion	14-bp del	Frameshift	Not mentioned
24		Exon 7	Nonsense mutation	nt 984C > G	Y328X	Not mentioned
25		Exon 8	Nucleotide deletion	9-bp del	Splicing error	Not mentioned
26		Exon 11	Missense mutation	nt 1551C > G	F517L	Not mentioned
27 Gu et al.	1998	Exon 1	Nucleotide insertion	nt 91 11-bp ins	Frameshift	Synkinesia
28 O'Neill et al.	1998	Intron 5	Nucleotide change	IVS 5-1G > A	Splicing error	Mental disturbance
29 Weissörtel et al.	1998	All exons	Contiguous gene syndrome			Mental disturbance
30 Maya-Núñez et al.	1998a	Exon 1–3	Contiguous gene syndrome			Not mentioned
31 Maya-Núñez et al.	1998b	Exon 3–5	Exonic deletion		In frame	Synkinesia
32		Exon 11	Missense mutation	nt 514G > A	E514K	None
33 Maya-Núñez et al.	1999	None	Contiguous gene syndrome			Not mentioned
34 Izumi et al.	1999	Exon 1	Nonsense mutation	nt 196C > T	Q66X	None
35 Ours	2000	Exon 5–10	Exonic deletion		Frameshift	None

KS, Kallmann syndrome; nt, nucleotide; del, deletion; ins, insertion

More than 34 different mutations of *KAL1* have been reported to date (Table 1) (Bick et al. 1992; Hardelin et al. 1992; Meindl et al. 1993; Hardelin et al. 1993a,b; Bouloux et al. 1993; Klink et al. 1994; Martul et al. 1995; Parenti et al. 1995; Quinton et al. 1996; Georgopoulos et al. 1997; Gu et al. 1998; O'Neill et al. 1998; Weissörtel et al. 1998; Maya-Núñez et al. 1998a,b and 1999; Izumi et al. 1999). Both of the patients described here had hypogonadism and hyposmia (the two hallmarks of KS), but they had no other neurological symptoms. Although a mental or intellectual disturbance was described in the original report of KS (Kallmann et al. 1944), recent analyses of the genotype-phenotype relationship of this syndrome have shown that KS patients with mental disturbance had large deletions on Xp22.3 that extended beyond *KAL1* (Table 1). In contrast, almost all of the patients with mutations restricted to the *KAL1* locus appeared to be free of mental disturbance. Therefore, another gene located close to *KAL1* was speculated to be responsible for the mental disturbance (Prager and Braunstein 1993).

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