BRIEF REPORT

Kallmann Syndrome: Somatic and Germline Mutations of the Fibroblast Growth Factor Receptor 1 Gene in a Mother and the Son

Naoko Sato, Kenji Ohyama, Maki Fukami, Michiyo Okada, and Tsutomu Ogata

Department of Endocrinology and Metabolism, National Research Institute for Child Health and Development (N.S., M.F., M.O., T.O.), Tokyo 157-8535, Japan; and Interdisciplinary Graduate School of Medicine and Engineering Sciences, University of Yamanashi (K.O.), Yamanashi 409-3898, Japan

Context: Although Kallmann syndrome (KS) caused by heterozygous loss of function mutations of the fibroblast growth factor receptor 1 gene (FGFR1) is occasionally associated with characteristic features, such as dental agenesis and cleft palate, FGFR1 mutations remain unidentified in several KS patients with such characteristic features.

Subjects and Methods: We examined a 14-yr-old Japanese boy with hypogonadotropic hypogonadism, olfactory dysfunction, and dental agenesis and his fertile mother with olfactory dysfunction and dental hypogonadotropic hypogonadism. Direct sequencing was performed for FGFR1 using leukocyte genomic DNA from the proband and leukocyte and nail genomic DNA from the mother. To examine a possible somatic mutation, a specific forward primer was designed to introduce a Bst XI site into the normal allele only, and nested PCR amplification, followed by Bst XI digestion, was carried out three times with different reverse primers.

Results: After standard PCR amplifications, a heterozygous 2-bp deletion at exon 10 (1317_1318delTG) is predicted to cause a frameshift at the 439th codon for serine and resultant termination at the 461st codon (S439fsX461), was identified in the proband, but was not found in the mother. After selective amplification of the mutant allele, this deletion was detected in nail DNA, but not in leukocyte DNA, from the mother.

Conclusion: The results suggest that the 2-bp deletion took place as a somatic mutation in the mother and was transmitted to the boy because of germline mosaicism. Such a somatic mutation occurs in some apparently FGFR1 mutation-negative KS patients with dental agenesis. (J Clin Endocrinol Metab 91: 1415–1418, 2006)

Patients and Methods

The proband of this family was a boy who was referred to Yamanashi University Hospital because of lack of pubertal development at 14 yr and 9 months of age. He exhibited a eunuchoid habitus with no pubertal development (pubic hair, Tanner stage 1; genitalia, Tanner stage 1). The penis was 2.8 cm long, and the testes were 2 ml in volume and palpable within the scrotum bilaterally. His height was 145.6 cm (–1.8 sd), and his weight was 57.8 kg (+0.2 sd). The annual growth data obtained at school indicated that his height remained around –0.2 sd in childhood and gradually decreased to –1.8 sd because of no pubertal growth spurt. His bone age was assessed as 12.5 yr by the TW-2 method standardized for the Japanese. A GnRH test (100 μg/m2 bolus iv; blood sampling at 0, 30, 60, 90, and 120 min) showed grossly age-appropriate serum gonadotropin values [basal LH, 0.7 IU/liter (normal range, 1.5–6.0 IU/liter); peak LH, 9.4 IU/liter (5.7–18.5 IU/liter); basal FSH, 2.9 IU/liter (1.5–6.0 IU/liter); peak FSH, 11.0 IU/liter (7.0–14.0 IU/liter)], whereas a human chorionic gonadotropin test (3000 IU/m2 dose; im, for 3 consecutive days; blood sampling on d 1–4) revealed a severely compromised serum basal testosterone (T) value [7.8 ng/dl (0.2 nmol/liter); normal range, 100–600 ng/dl (3.4–20.8 nmol/liter)] and subnormal T response [225 ng/dl (7.8 nmol/liter); >300 ng/dl (>10.4 nmol/liter)]. Furthermore, he had no sense of smell, and magnetic resonance imaging

cells in an affected individual. Furthermore, if the somatic mutation occurs at an early stage before the separation of germline cells from somatic cells, it can be transmitted to the offspring in its complete form because of germline mosaicism (8). In this study we report on a Japanese family in which a paternal somatic mutation of FGFR1 was transmitted to her son as a germline mutation.

Abbreviations: FGFR1, Fibroblast growth factor receptor 1; HH, hypogonadotropic hypogonadism; KS, Kallmann syndrome; T, testosterone.
delineated mildly hypoplastic olfactory bulbbs. Thus, he was diagnosed as having KS. He also had agenesis of eight teeth (first upper and lower molars, upper premolar teeth, and lower canine teeth), although he was free from other features reported in KS, such as mirror movement, renal aplasia, cleft palate, high arched palate, and perceptive deafness. He was treated with T enanthate (125 mg/month, im) from 15 yr and 4 months of age. On the last examination at 17 yr and 7 months of age, he was 169.3 cm in height (−0.2 sd) and 63.4 kg in weight (+0.2 sd), and manifested secondary sexual development (pubic hair, Tanner stage 4; genitalia, Tanner stage 3), although his testes remained 2 ml in volume bilaterally.

The 47-yr-old mother also lacked a sense of smell and had agenesis of six teeth (first upper and lower molars and lower canine teeth). She had normal stature (160.0 cm; +0.4 sd) and exhibited full secondary sexual development. In the follicular phase, basal serum LH was 3.1 IU/liter (normal range, 1.8–7.6 IU/liter), FSH was 3.7 IU/liter (normal range, 5.2–14.4 IU/liter), and estradiol was 70.1 pg/ml [257 pmol/liter; normal range, 20–120 pg/ml (73.4–440 pmol/liter)]. Her menarche occurred at 13.6 yr of age (normal range, 9.75–14.75 yr) and was followed by regular menses of 34-day cycles (normal cycle periods, 24–35 d) (9). She gave birth to four children after uncomplicated term pregnancies and deliveries and fed them with artificial milk. Her menses resumed more than 1 yr after each delivery, although menses usually resume at approximately 6 wk postpartum in nonbreast feeding women (10). However, no data were available for factors influencing the postpartum amenorrheic periods, such as the postpartum weight change, the degree of exercise, and the pituitary-gonadal function status.

The father and the three children (a 20-yr-old boy, a 16-yr-old boy, and a 13-yr-old girl) had age-appropriate secondary sexual development and normal sense of smell. Furthermore, dental examination showed no abnormality in the four family members. Allegedly, the maternal parents also had no discernible abnormal features.

**Molecular analysis of FGFR1**

This study has been approved by the institutional review board committee at the National Center for Child Health and Development. After obtaining written informed consent, leukocyte genomic DNA of the proband and the mother was PCR amplified for the coding exons of FGFR1 (exons 2–18), and the PCR products were subjected to direct sequencing from both directions on a CEQ 8000 autosequencer (Beckman Coulter, Fullerton, CA). The primer sequences and PCR conditions have been described previously (5). Consequently, a heterozygous 2-bp deletion at exon 10 (1317_1318delTG), which is predicted to cause a frameshift at the 439th codon for serine and resultant termination at the 461st codon (S439fsX461), was identified in the proband (Fig. 1A). This mutation was confirmed by sequencing the subcloned normal and mutant alleles (TOPO TA Cloning Kit, Invitrogen Life Technologies, Inc., Carlsbad, CA). By contrast, this 2-bp deletion was not detected in the mother (Fig. 1B).

Thus, the possibility of a somatic mutation was examined in the mother. For this purpose, approximately 1.3 μg of genomic DNA was extracted from the 10 fingernails that were left uncut for two wk, using SMITEST EX-R&D kit (Genome Science Laboratories Co., Ltd., Fuku-shima, Japan) (11). To avoid contamination of the proband’s tissue such as a piece of nail trapped in the nail cutter or skin scratched by the maternal nails, the maternal nails were obtained with a new nail cutter after careful washing with a brush at the Hospital. Then, 0.1 μg of nail genomic DNA was amplified for the region harboring the 2-bp deletion mutation by PCR of 35 cycles, with equipments that were never used for the DNA analysis of the proband, to avoid contamination of the proband’s DNA. Direct sequencing of the PCR products, however, failed to detect the mutation.

Next, a selective amplification of the mutant allele was attempted for

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**Fig. 1. Sequence analysis of FGFR1 in the proband and the mother. A, Electropherograms obtained by the standard amplification for the leukocyte genomic DNA of the proband. A heterozygous 2-bp deletion at exon 10 (1317_1318delTG) has been indicated by the direct sequencing, and confirmed by the subsequent sequencing of the subcloned normal and mutant alleles. This mutation is predicted to cause a frameshift at the 439th codon for serine and resultant termination at the 461st codon (S439fsX461). B, An electropherogram obtained by the standard amplification for the leukocyte genomic DNA of the mother. The nucleotide sequence is normal with no trace of the 2-bp deletion mutation identified in the proband. C, Electropherograms obtained by the selective amplification of the mutant allele for the nail genomic DNA of the mother. The reverse sequence is shown. A heterozygous 2-bp (CA) deletion corresponding to the 1317_1318delTG at exon 10 has been delineated by the direct sequencing, and confirmed by the subsequent sequencing of the subcloned normal and mutant alleles. Note that the “C” nucleotide corresponding to the “G” nucleotide that should have been dropped after the amplification with the specific forward primer (see Fig. 2) is deleted from the normal and the mutant alleles.
the leukocyte and the nail genomic DNA. Because there was no restriction enzyme that specifically digest the normal allele, a specific forward primer was designed to introduce a BstXI site into the normal allele only, and nested PCR amplification followed by BstXI digestion was carried out three times with different reverse primers (Fig. 2). Each run of nested PCR was performed for 35 cycles. Subsequently, sequence analysis was performed for the final PCR products from a reverse direction, delineating the 2-bp deletion mutation in the nail DNA (Fig. 1-C), while the mutation was still undetected in the leukocyte DNA. Other tissues such as hair, buccal cell swabs, and skin fibroblasts were not examined, because the mother hoped us to examine the nail as a first step, and to analyze other tissues when no mutation was identified in the nail.

**Results and Discussion**

The heterozygous 2-bp mutation was identified for the leukocyte DNA by the standard direct sequencing in the proband, whereas it was revealed in the nail, not leukocyte, DNA after the selective amplification of the mutant allele in the mother. Because contamination of the proband’s tissue or DNA to the maternal sample was prevented as much as possible, it is likely that the 2-bp deletion mutation took place as a somatic mutation involving the germ cells in the mother and was transmitted to the proband as a germline mutation. Furthermore, the results indicate the usefulness of PCR-based selective amplification of the mutant allele and the importance to analyze plural tissues in the detection of a somatic mutation. In this regard, because the 2-bp deletion mutation was identified in the nail DNA and the mother had dental agenesis, it may relatively be prevalent in the ectodermal tissues. The mutant allele, however, would be infrequent even in the nails, because it was undetected after the standard PCR amplification. At the same time, the mutant allele, though it was identified after the nested PCR, would not be extremely rare, because the hybridization efficiency of the specific forward primer missing the “G” nucleotide would be low for both the normal and the mutant alleles (Fig. 2).

Because the 2-bp deletion mutation leads to the frameshift and resultant premature termination, it should be a loss-of-function mutation, as has been indicated in KS (4–7). Thus, clinically discernible HH, olfactory dysfunction, and dental agenesis of the proband would primarily be ascribed to the heterozygous germline mutation of FGFR1 (4, 6, 7). For HH, although the results of the GnRH test were grossly normal, endogenous GnRH secretion would have more or less been attenuated in the proband. In this regard, the endocrine data would be consistent with the previous finding that human chorionic gonadotropin-stimulated T value is more useful than GnRH-stimulated gonadotropin value in the diagnosis of HH in prepubertal boys (12). Furthermore, the maternal fertile phenotype may be compatible with the somatic mutation, because clinical features can be mitigated by the presence of mutation negative cells in the target tissues (8). Indeed, her menarchial age and menstrual cycles remained

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**FIG. 2**. The methods for the identification of the somatic mutation in the mother. A, The F0 primer sequence used for the induction of a restriction enzyme site into the normal allele preserving the “TG” nucleotide (italicized). The primer has been designed to drop the “G” nucleotide (boldfaced) after PCR amplification. As a consequence, a BstXI site (underlined) is created in the normal allele, but not in the mutant allele. B, The selective amplification of the mutant allele in the mother. The amplification consists of three steps: 1) the first PCR with F0 and R546 primers for the genomic DNA, followed by the BstXI digestion; 2) the second PCR with F0 and R422 primers for the products of the first PCR, followed by the BstXI digestion; and 3) the third PCR with F0 and R248 primers for the products of the second PCR, followed by the BstXI digestion. Consequently, the normal allele (shown as horizontal lines, with dots indicating the BstXI site) is gradually eliminated, and the mutant allele (shown as horizontal lines without dots) is gradually amplified. The final products have been sequenced from the reverse direction. The sequence of each primer is shown in the box, together with the annealing temperature (AT) and the product size.
within the normal range, while it is uncertain whether her prolonged postpartum amenorrheic periods are related to the somatic mutation. However, the phenotypic spectrum in germline FGFR1 mutation positive patients is known to be variable, including the typical KS phenotype, olfactory dys-function only phenotype, and apparently normal phenotype (4–7). In addition, FGFR1 and anosmin-1 encoded by KAL1 are likely to interact in FGF signaling involved in the development of olfactory bulbs (the target regions in KS) (4, 6, 13), and the local concentration of anosmin-1 should be higher in females than in males, because KAL1 partially escapes X-inactivation (3). Such a wide phenotypic variability and an advantageous factor in females may also account for the preserved fertility in the mother.

The present data would provide a useful implication for the molecular diagnosis. To date, FGFR1 mutations have not been identified in several KS patients with cleft palate and/or dental agenesis (5). Such patients might also have somatic mutations of FGFR1 that could not be detected in leukocyte DNA by the standard sequencing method.

Acknowledgments

Received October 12, 2005. Accepted January 9, 2006.
Address all correspondence and requests for reprints to: Dr. Tsutomu Ogata, Department of Endocrinology and Metabolism, National Research Institute for Child Health and Development, Tokyo 157-8535, Japan. E-mail: tomogata@nch.go.jp.

This work was supported by Child Health and Development from the Ministry of Education, Science, Sports, and Culture (16086215). Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Health, Labor, and Welfare (17C-2), a grant from the Kawano Masanori Memorial Foundation for Promotion of Pediatrics, and a grant from the Kawano Japan. E-mail: tomogata@nch.go.jp.

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