Kallmann syndrome with a Tyr113His PROKR2 mutation

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Abstract
Rational: Kallmann syndrome (KS) is a genetic gonadotropin-releasing hormone deficiency associated with hyposmia or anosmia and characterized by various modes of inheritance.

Patient concerns: A 16-year-old male did not reach puberty and was associated with hypogonadotropic hypogonadism and anosmia. His magnetic resonance imaging of brain revealed the absence of the olfactory bulb.

Diagnosis: His karyotype was 46 XY. Sanger sequencing of the KAL1 gene revealed no mutations. Diagnostic exome sequencing identified a prokineticin-receptor 2 (PROKR2) gene variant, c.337T>C (p.Tyr113His), previously reported to be a pathogenic mutation; we confirmed the presence of the mutation via Sanger sequencing of the coding exons of PROKR2. His apparently unaffected mother and sister, but not his father, were heterozygous for the PROKR2 Tyr113His mutation.

Lessons: This work advances our understanding of the role played by PROKR signaling and the mode of inheritance of the gene in patients with KS.

Abbreviations: GnRH = genetic gonadotropin-releasing hormone, KS = Kallmann syndrome, PROKR2 = prokineticin-receptor 2.

Keywords: exome sequencing, Kallmann syndrome, prokineticin-receptor 2.

1. Introduction
Kallmann syndrome (KS), characterized by hypogonadotropic hypogonadism and anosmia, commences in embryonic life; neurons that synthesize gonadotrophin-releasing hormone (GnRH) fail to migrate from the olfactory placode to the frontonasal region of the brain.[1,2] KS is genetically heterogeneous; several causative genes have been identified, as have various modes of inheritance.[3] Despite recent advances in genetics, the relevant pathogenic genes of KS have been identified in <30% of cases.

In the present study, we used diagnostic exome sequencing to identify the genetic defect in a patient with KS. Herein, we report the phenotypic features and inheritance pattern of a missense prokineticin-receptor 2 (PROKR2) mutation within the family of that patient.

2. Presenting concerns
A Korean male presented at the age of 16 years because of the absence of puberty. He was the first child of unrelated, healthy parents, and had been born after a full-term pregnancy.

3. Clinical findings
At presentation, he was 171 cm in height and weighed 86 kg (body mass index, 29 kg/m²). He exhibited anosmia, small testes...
(testis volume, 4 mL), and a stretched penile length of 20 mm. Anosmia was confirmed by application of the quantitative smell test (the Korean Version of the Sniffin Sticks Test; KVSS Test II).[4] The laboratory test showed low level of luteinizing hormone and follicular stimulating hormone and testosterone also (Table 1). His magnetic resonance imaging of brain revealed the absence of the olfactory bulb (Fig. 1).

4. Diagnostic focus and assessment

His karyotype was 46 XY. Sanger sequencing of the KAL1 gene revealed no mutations. We performed diagnostic exome sequencing of about 62,000 exons of 4813 genes using the TruSight One Panel (Illumina Inc., San Diego, CA) running on the Illumina MiSeq platform. The mean coverage was 91-fold; 98% of the targeted bases were read >10 times. Single-nucleotide polymorphisms and short indel candidates identified by ANNOVAR were filtered using the dbSNP129 and “1000 Genomes Project” software. A heterozygous missense variant in PROKR2 (NM_144773.2:c.337T>C; p.Tyr113His) was identified, validated by Sanger sequencing, and confirmed to be pathogenic by the Sorting Intolerant From Tolerant and Polymorphism Phenotyping software, v. 2 (https://genetics.bwh.harvard.edu/pph2/).[5] No mutation was identified in any other KS-related gene (e.g., KAL1, FGFR1, FGF8, CHD7, or PROK2). The identical heterozygous missense variant was identified in his younger sister and mother, but not his father, upon Sanger sequencing of PROKR2 (Fig. 2). His mother and younger sister attained menarche at the ages of 13 and 12 years, respectively; their sense of smell was normal and they had measurable estradiol levels (Table 1). His sister exhibited normal breast development exceeding Tanner stage V (Fig. 3).

5. Discussion

We used diagnostic exome sequencing to seek a genetic cause of KS, and identified a relevant heterozygous PROKR2 substitution (c.337T>C; p.Tyr113His).

| Basal hormone value | Patient | Mother | Sister |
|---------------------|---------|--------|--------|
| LH, IU/L            | <0.07   | NA     | NA     |
| FSH, IU/L           | 0.32    | NA     | NA     |
| Testosterone, ng/mL | 0.05    | <0.0   | 0.29   |
| Estradiol, pg/mL    | 15.10   | 20.23  | 161.62 |
| GH, ng/mL           | N       | 0.22   | 0.08   |
| TSH, IU/L           | 1.17    | 0.42   | 1.01   |
| FT4, ng/dL          | 1.15    | 1.18   | 1.08   |
| ACTH, pg/mL         | 46.55   | 2.36   | 39.62  |
| Cortisol, μg/dL     | 10.74   | <0.50  | 11.75  |

The proband has low level of luteinizing hormone, follicular stimulating hormone, and testosterone but has normal level of other hormones. The family members of proband have no significance in level of hormones. ACTH = adrenocorticotropic hormone, FSH = follicular stimulating hormone, FT4 = free thyroxine, GH = growth hormone, LH = luteinizing hormone, NA = not available, TSH = thyroid-stimulating hormone.

Figure 2. Family electropherograms revealing the c.337T>C PROKR2 mutation. The proband, his mother, and his sister were heterozygous for the nonsynonymous mutation. His father did not have the variant. From top to bottom: proband, father, mother, and sister.
Of the several genetic loci known to be involved in KS, loss-of-function mutations compromising signaling via PROK2-PROKR2 are known to contribute to a KS phenotype.\textsuperscript{[5,6]} PROKR2 is primarily localized in the central nervous system, and is abundantly expressed in the olfactory bulbs. Matsumoto et al.\textsuperscript{[7]} showed that Prokr\textsuperscript{-/-} knockout mice had a KS-like phenotype: exhibiting early hypoplasia of the olfactory bulbs and severe reductions in the sizes of the reproductive organs in both sexes, with an absence of neuroendocrine GnRH cells in the hypothalamus. Mutations (such as Tyr113His) located near the extracellular receptor loop in a female patient with KS, and found that HEK-293 cells with the mutation exhibited reductions in the early growth responses to I-luciferase, Ca\textsuperscript{2+} influx, and receptor signaling. However, neither the clinical phenotype nor inheritance mode of the mutation has been precisely defined.

Although the Tyr113His missense mutation was pathogenic in our case, the mutation was also present in phenotypically unaffected family members (the mother and sister). Variation in expressivity and incomplete penetrance within and across families has been reported in certain families sharing identical heterozygous mutations in PROK2 and PROKR2.\textsuperscript{[5,6,8–10]} Although digenic or oligogenic inheritance of KS has been suggested,\textsuperscript{[11,12]} the contributions of these modes of inheritance remain uncertain. Our present case had a severe GnRH deficiency and olfactory bulb dysgenesis. However, the presence of asymptomatic family carriers (the mother and sister) of the PROKR2 heterozygous mutation raised the possibility that our case had another unknown genetic defect.

We found no additional mutations upon exome sequencing of the candidate KS genes (KAL1, FGFR1, PROK2, CHD7, and FGF8). This finding is consistent with those of several previous studies on patients with KS having heterozygous PROKR2 point mutations but without mutations in any other candidate gene upon Sanger sequencing.\textsuperscript{[5,6]} Two large cohort studies on patients with KS identified 13 PROKR2 mutations differing in zygosity (heterozygous, homozygous, and compound heterozygous).\textsuperscript{[5,6]} Incomplete mutation penetrance may explain the variation in the inheritance mode in patients with heterogeneous or sporadic KS. Further genetic studies in such patients are needed to establish the genetic pathogenesis of KS.

The practical difficulties encountered when attempting to implement a genetic testing strategy would include genetic heterogeneity, the small number of cases, and the fact that the genetic cause is not yet fully established. However, application of next-generation sequencing could facilitate the genetic diagnosis of hypogonadotropic hypogonadism, allowing counseling to become personalized.\textsuperscript{[13]} Our study suggests that mutation of the PROKR2 gene should be considered in KS patients; this would advance our knowledge on the role played by PROKR signaling and the mode of inheritance of the mutation in patients with KS.

6. Informed consent
We obtained written informed consent from the patient’s parent to conduct genetic tests.

References
\textsuperscript{[1]} Schwanzel-Fukuda M, Pfaff DW. Origin of luteinizing-hormone-releasing hormone neurons. Nature 1989;338:161–4.
\textsuperscript{[2]} Kallmann F. The genetic aspects of primary eunuchoidism. Am J Ment Defic 1944;48:203–36.
\textsuperscript{[3]} Bianco SD, Kaiser UB. The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. Nat Rev Endocrinol 2009;5:569–76.
\textsuperscript{[4]} Hong S-M, Park I-H, Kim K-M, et al. Relationship between the Korean Version of the Sniffin’ Stick Test and the T&T Olfactometer in the Korean Population. Clin Exp Otorhinolaryngol 2011;4:184–7.
\textsuperscript{[5]} Cole LW, Sids Y, Zhang C, et al. Mutations in prokineticin 2 and prokineticin receptor 2 genes in human gonadotrophin-releasing hormone deficiency: molecular genetics and clinical spectrum. J Clin Endocrinol Metab 2008;93:3551–9.
\textsuperscript{[6]} Dodé C, Teixeira L, Levilliers J, et al. Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. PLoS Genet 2006;2:e175.
\textsuperscript{[7]} Matsumoto S-I, Yamazaki C, Masumoto K-h, et al. Abnormal development of the olfactory bulb and reproductive system in mice lacking prokineticin receptor PKR2. Proc Natl Acad Sci U S A 2006;103:4140–5.
\textsuperscript{[8]} de Roux N, Young J, Misrahi M, et al. A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. N Engl J Med 1997;337:1597–603.
\textsuperscript{[9]} Salenave S, Chanson P, Bry H, et al. Kallmann’s syndrome: a comparison of the reproductive phenotypes in men carrying KAL1 and FGFR1/KAL2 mutations. J Clin Endocrinol Metab 2008;93:758–63.
\textsuperscript{[10]} Sarfati J, Guiochon-Mantel A, Rondard P, et al. A comparative phenotypic study of kallmann syndrome patients carrying monoallelic and biallelic mutations in the prokineticin 2 or prokineticin receptor 2 genes. J Clin Endocrinol Metab 2010;95:639–49.
\textsuperscript{[11]} Pitteloud N, Quntion R, Pearce S, et al. Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. J Clin Invest 2007;117:457–63.
\textsuperscript{[12]} Quaynor SD, Kim H-G, Cappello EM, et al. The prevalence of digenic mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome. Fertil Steril 2011;96:1424–30, e6.
\textsuperscript{[13]} Lu JT, Campeau PM, Lee BH. Genotype-phenotype correlation—promiscuity in the era of next-generation sequencing. N Engl J Med 2014;371:593–6.