Biallelic PROKR2 variants and congenital hypogonadotropic hypogonadism: a case report and a literature review

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Abstract. Congenital hypogonadotropic hypogonadism (CHH) is a rare disorder that causes gonadotropin-releasing hormone (GnRH) deficiency and sexual immaturity. CHH may accompany an abnormal sense of smell (Kallmann syndrome, KS) or no such manifestation (normosmic-CHH). This unusual combination of manifestations is explained by the fact that GnRH neurons originate in the olfactory placode and migrate to the forebrain during embryogenesis. We describe the case of a 31-year-old man with normosmic-CHH, who also had obesity, type 2 diabetes and intellectual disability. He was noticed to have sexual immaturity (small testes with no pubic hair) at age 20 years, when diabetic ketoacidosis developed. Basal and GnRH-stimulated levels of LH (1.0→12.0 IU/L) and FSH (1.9→6.1 IU/L) were detectable but low. The results of the T&T olfactometer and the Alinamin test were definitely normal, with an anatomically normal olfactory system on MRI. Sequencing of 22 CHH-related genes was performed, and compound heterozygous PROKR2 variants were identified: one was a previously known loss-of-function variant (p.Trp178Ser) and the other was a nonsense variant (p.Trp212*). Through a literature review, we found 22 patients (including our patient) with CHH due to biallelic PROKR2 variants, which led us to recognize that most of the patients (86%) were diagnosed with KS. Clinical observations in this study indicate that, even though they have CHH, biallelic PROKR2 variant carriers may have a normal olfactory system as well as presumably normal migration of GnRH neurons. This suggests that the PROK2-PROKR2 pathway affects the function of GnRH neurons after their migration.

Key words: Hypogonadotropic hypogonadism, Normosmia, PROKR2, Mutation

CONGENITAL HYPOGONADOTROPIC HYPOGONADISM (CHH) is a rare disorder characterized by a lack of normal pulsatile gonadotropin-releasing hormone (GnRH) secretion, thereby leading to sexual immaturity and infertility [1]. CHH may present with either gonadotropin deficiency only, or may be accompanied by a dysfunctional sense of smell (anosmia or hyposmia). The latter condition is referred to as Kallmann syndrome (KS). In patients with KS, other congenital abnormalities such as cleft lip/palate, dental defects, ear abnormalities, kidney malformations, mirror movements of hands (manual synkinesis) and skeletal abnormalities can be seen.

This unique combination of GnRH deficiency and olfactory abnormalities can be attributed to the developmental process of GnRH neurons. GnRH neurons are unusual neuroendocrine cells that originate in the olfactory placode (outside the central nervous system), and migrate into the forebrain during embryogenesis [2]. Therefore the impaired development of olfactory neuron can affect the migration of GnRH neurons [3]. Historically, the association of hypogonadism and anatomical abnormalities in the olfactory system (later became known as KS) was first described in 1856 by Maestre de San Juan through an autopsy of a 40-year-old man [4]. Franz Josef Kallmann characterized this association as a hereditary condition in 1944 [5]. Segregation analysis...
showed several modes of inheritance, including X-linked [6], autosomal dominant [7] and autosomal recessive [8].

With the application of DNA sequencing technology in human genetics research, ANOS1 (formerly KAL1) was first identified as the gene responsible for X-linked KS [9]; it was followed by the identification of FGFR1, as the gene responsible for autosomal dominant KS [10]. In 2006, knockout mice of Prok2, which encodes prokineticin 2 [11], and its receptor (Prokr2) [12], were shown to have KS-like phenotypes. Shortly thereafter, PROKR2 was identified as the first gene responsible for autosomal recessive KS [13]. These initial findings were followed by the identification of genetic variants not only among KS but also in CHH cases with normal olfaction (normosmic-CHH), thereby expanding their phenotypic spectrums [14]. To date, more than 30 CHH-associated genes have been identified (reviewed in Ref. [1]); it is also known that genetic variants of two or more genes may be simultaneously observed in one individual [15, 16] (i.e., oligogenicity). Currently, CHH is recognized as a group of diseases in which a single or a small number of genes influence its pathogenesis. Nonetheless, knowledge about the relationship between affected genes and clinical phenotypes is still fragmented; thus, high-quality clinical information may still be needed. Here, we describe the first Japanese patient with CHH due to biallelic PROKR2 variants. We also discuss the role of the PROK2–PROKR2 pathway in the reproductive axis based on our clinical observations.

**Subject and Methods**

**Case report**

The patient was a 31-year-old Japanese man who was born at 41 weeks of gestation after an uncomplicated pregnancy and delivery. His parents were non-consanguineous and phenotypically normal with no history of receiving assisted reproductive technology. Family history included multiple individuals with obesity and diabetes in the pedigree (Fig. 1A; I-2, II-3, II-4, III-2). According to the mother, there were no abnormalities in the puberty of patient’s brother, although no endocrinological evaluation was performed. During early childhood, the patient presented with obesity and intellectual disability. His school performance was reportedly extremely poor. At age 13 years, glycosuria was detected via an annual school health check-up. He had high fasting plasma glucose (252 mg/dL) and HbA1c (10.4%, NGSP) levels; he was diagnosed with type 2 diabetes. Glycemic control was improved by diet therapy at 1,600 kcal/day. His primary physician documented micropenis at this point; however, no further evaluation was performed. Three years later, treatment with sulfonylurea was started, because outpatient diet therapy could no longer be continued.

At age 20 years, diabetic ketoacidosis was noted upon discontinuing sulfonylurea treatment, thereby leading to hospital admission. At this point, a lack of secondary sexual characteristics was observed. The patient was 173.5 cm tall and weighed 94.5 kg (BMI 31.4 kg/m²). Vocal changes have not yet occurred; a micropenis with no pubic hair was also observed. His testes were small (right 5 mL, left 7 mL; reference 12.5–20 mL). Testicular biopsy revealed few spermatozoa in the section (Johnsen’s score, 8 [17]). He had a low plasma testosterone level (0.5 ng/mL; reference 2.5–14), and low but detectable basal and GnRH-stimulated gonadotropin levels (LH 1.0–12.0 IU/L, FSH 1.9–6.1 IU/L); however, the response of gonadotropins to 100 mg clomiphene for 7 days was abrogated (LH <0.1 IU/L, FSH <0.1 IU/L). Serum levels of other anterior pituitary hormones were normal (data not shown); he was diagnosed with CHH. The Wechsler Adult Intelligence Scale-R examination showed low IQ scores (verbal 23, performance 19, full-scale 42).

We evaluated his olfactory function using a T&T olfactometer [18] and an intravenous olfaction test with thiamine (the Alinamin test). The T&T olfactometer test, which is equivalent to the University of Pennsylvania Smell Identification Test [19], is a standard olfactory sensitivity test in Japan. Five types of standard odors were assessed to determine the detection and recognition thresholds; the patient demonstrated normal results, with the exception of poor recognition of skatole (smell of vegetable garlic) (Fig. 1B). For the intravenous olfaction test, the latent time was normal (8 s, normal range, 7–8). A brain MRI showed normal olfactory sulcus (Fig. 1C).

**Genetic analysis**

We obtained written informed consent from the patient and his father for molecular studies. The study protocol was approved by the Ethics Committee of Keio University School of Medicine. Genomic DNA was isolated from the patient’s leukocytes and his father’s saliva using standard techniques. We performed next-generation sequencing (NGS) using a MiSeq instrument (Illumina, Inc., San Diego, CA, USA) with the SureSelect protocol (Agilent Technologies, Santa Clara, CA, USA) as previously described [20]. A total of 22 CHH-associated genes were tested as follows: ANOS1, CHD7, FGFR8, FGFR1, FSHB, GLI2, GNRH1, GNRHR, HESX1, KISS1, KISS1R, LHB, LHX4, OTX2, PAX6, POU1F1, PROK2, PROKR2, PROP1, SOX2, TAC3 and TACR3. In this study, we set two thresholds for allele frequency (AF) to define rare variants considering the prevalence of CHH.
[21]: (A) population AF less than 0.001 for genes responsible for autosomal dominant inheritance or X-linked inheritance; and (B) population AF less than 0.005 for genes responsible for autosomal recessive inheritance. Population AF was searched with gnomAD v2.1.1 (https://gnomad.broadinstitute.org/) and 8.3KJPN (https://jmorp.megabank.tohoku.ac.jp/), where the highest values were used accordingly. The presence of rare variants detected by NGS was confirmed by PCR-based Sanger sequencing in the patient and his father.

Oligonucleotide array comparative genomic hybridization was performed with SurePrint G3 Human CGH Microarray 4× 180K (Agilent, Santa Clara, CA, USA) according to the manufacturer’s instructions.

**Literature review of biallelic PROKR2 variant carriers**

To find information on biallelic PROKR2 variant carriers from the literature, we searched the Human Gene Mutation Database Professional (Version_2021.3). A total of 88 cases with at least one allele of PROKR2 variants were reported in 33 studies. Upon further inspection, we extracted 22 patients with biallelic PROKR2 variants (i.e., homozygous or compound heterozygous) reported in eight studies [22-29]. To find PROKR2 variant carriers that were not registered in the Human Gene Mutation Database, two authors (C.S. and S.N.) independently searched PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Google Scholar (https://scholar.google.com/) to find relevant articles published by November 2021. We collected information about sex, clinical diagnosis (KS or normosmic-CHH), PROKR2 variant(s), evaluation of olfaction, MRI findings, serum levels of LH and FSH (basal and GnRH-stimulated) and BMI.

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**Fig. 1** Clinical and genetic findings of the patient. (A) A pedigree of the patient. Squares and circles represent male and female, respectively. An affected individual with CHH (proband, indicated by an arrow) is indicated with a solid symbol. (B) Results of the T&T olfactometer test of the patient. The normal range is shown as a shaded area. Higher scores indicate higher olfactory threshold. (C) An MRI image of a coronal section of the brain of the patient. Olfactory sulcus (OS) is indicated by arrows. (D) Partial electropherograms of PCR products amplified from PROKR2 using genomic DNA of the patient. Observed nucleotide changes are indicated by arrows. (E) Sequence readings generated by NGS were visualized with Integrative Genomics Viewer. The mutually exclusive observation of reads with p.Trp212* and p.Trp178Ser indicates that they were in the compound heterozygous state. A common polymorphism rs3746682 is detected in the reads with p.Trp178Ser.
Results

Genetic analysis

To reveal the molecular basis of normosmic-CHH in the patient, we performed NGS-based comprehensive genetic screening for CHH. Using this analysis, we identified two rare amino acid sequence-altering variants, both of which were in PROKR2 in a heterozygous state (Fig. 1D). One was a missense variant (c.533G>C, p.Trp178Ser), which has been reported in two unrelated patients with CHH [22]; it was registered in gnomAD v2.1.1 (AF = 0.002807 in East Asian) and 8.3KJPN (AF = 0.0012 in Japanese). The loss-of-function of Trp178Ser-PROKR2 was confirmed in vivo in a previous report [30]. The other was a nonsense variant (c.635G>A, p.Trp212*) that has not been reported in CHH patients but was registered in gnomAD v2.1.1 (AF = 0.00003266 in South Asian). The compound heterozygosity of the two variants was confirmed upon inspecting the NGS readings (Fig. 1E). The unaffected father was heterozygous for the p.Trp212* variant. The mother declined genetic analysis.

In the remaining 21 CHH-associated genes, no rare variants were detected in the targeted exons. Oligonucleotide array comparative genomic hybridization analysis revealed no significant copy number variation (data not shown).

Literature review of CHH cases due to biallelic PROKR2 variants

Through a literature review, we found a total of 21 patients (belonging to 17 families) with CHH that had biallelic PROKR2 variants. We summarized the genetic and clinical information of the 22 patients, including our patient (Table 1). The patients had a total of 18 distinct PROKR2 variants, including 14 missense and four truncating variants (i.e., nonsense and frameshift variants). No “hot spots” of missense variants were recognized (Fig. 2A); however, the locations of missense variants were more frequent in the seven-transmembrane domain than in the cytoplasmic tail or the extracellular portion. In the 22 variant carriers, the clinical diagnosis was KS in 86% and normosmic-CHH in 14% (Fig. 2B). There were more male than female patients. Seventeen of the 22 patients (77%) had missense variants in both alleles. Of the remaining five, four were compound heterozygotes for missense and truncating variants; one had a truncating variant in both alleles (Fig. 2B). No significant correlation between the above-mentioned genotype patterns (missense + missense, missense + truncating or truncating + truncating) and sense of smell (Table 1) or serum gonadotropin levels (Fig. 2C) were observed.

Discussion

In this study, we report the first Japanese patient with CHH due to biallelic PROKR2 variants. Our patient was characterized by a normal olfactory system and relatively mild hypogonadism. As shown in the literature review, normal olfaction is uncommon among biallelic PROKR2 variant carriers. Prior to this report, only two such patients have been reported [22, 29]; in both cases, detailed clinical information including olfactory test results, brain MRI findings or serum gonadotropin levels were unavailable. Furthermore, the pathogenicity of the two variants (p.Met111Arg and p.Thr340Ser) found in previously reported patients with normosmic-CHH has not been confirmed in vitro, thereby making it difficult to rigorously discuss the relationship between the variants and the disease. Our patient showed normal results for the T&T olfactometer, intravenous olfaction test and brain MRI.

It is unclear as to why our patient had an atypical phenotype in terms of olfaction. One possibility is the high residual activity of the PROKR2 variant proteins. The nonsense variant (p.Trp212*) found in our patient is seemingly non-functional. As for the other missense variant, p.Trp178Ser, it was reported to have a low ligand-dependent Ca\(^{2+}\) release capacity in vitro [30]; meanwhile, the signalling defect could be rescued by the action of chaperones that enhance the trafficking of the Trp178Ser-PROKR2 protein to the plasma membrane [31]. In addition, the compensation of PROK2-PROKR2 signalling by PROKR1 cannot be excluded, considering the expression of PROKR1 in the olfactory bulb [32] and in the arcuate nucleus of the hypothalamus [33]. Another possibility is the modification of CHH phenotypes by genetic variants other than PROKR2. It is well known that a subset of CHH patients has multile genetic variants in two or more genes [15, 16]. In this study, 22 CHH-associated genes were sequenced; the patient had no additional variants. For biallelic PROKR2 variant carriers with olfactory disturbance, similar NGS-based comprehensive screening has been reported in five studies [22, 24, 26, 27, 29]. However, none of the biallelic PROKR2 variant carriers had an additional variant, which may indicate that a defect of PROKR2 is sufficient to cause olfactory deterioration. Thus, there is no experimental evidence supporting the modification of olfactory or CHH phenotypes by other genes in the reported variant carriers.

Interestingly, our patient not only had normal olfaction, but also had relatively high serum gonadotropin levels compared with previously reported biallelic PROKR2 variant carriers (Fig. 2C). It is widely accepted that CHH in the PROK2-PROKR2 signalling defect is...
### Table 1  Summary of 22 patients with CHH due to biallelic PROKR2 variants

<table>
<thead>
<tr>
<th>Case ID</th>
<th>Family ID</th>
<th>Diagnosis</th>
<th>Sex</th>
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<th>PROKR2 Variants 2</th>
<th>Gq-activating capacity</th>
<th>Gq-activating capacity</th>
<th>Genotype Pattern</th>
<th>Other Analyzed Genes</th>
<th>Offactory Structure on MRI</th>
<th>Sense of Smell</th>
<th>BMI (kg/m²)</th>
<th>Serum LH (IU/L) basal → peak</th>
<th>Serum FSH (IU/L) basal → peak</th>
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</tbody>
</table>

1 Evaluation was based on Refs. 26 and 30. High, moderate and low capacities indicate >70%, 30–70% and <30% activity, respectively. 1 This report. Abbreviations: KS, Kallmann syndrome; n-CHH, normosmic congenital hypogonadotropic hypogonadism; NA, not available.
chiefly due to impaired migration of GnRH neurons. One possibility is that the migration of GnRH neurons is not sufficient to produce full gonadotrope function. However, the migration of olfactory neurons did not seem to be disturbed, suggesting that GnRH neurons may also migrate normally. Thus, biallelic PROKR2 variant carriers may have CHH even with normal migration. Instead of migration defect, post-migration modification of GnRH neurons may be related to mild gonadotropin deficiency in our patient. Our case suggests that PROK2-PROKR2 signalling plays a role other than GnRH neuron migration. In mice, the Prokr2 protein is expressed in the suprachiasmatic nuclei (SCN) of the hypothalamus, and is essential for the regulation of circadian behavior [34]. Considering that the involvement of SCN in GnRH/LH surge via neuronal projection has been well characterized in female mice (reviewed in Ref. [35]), it seems reasonable to speculate that PROK2-PROKR2 signalling in the SCN influences the function-ality of GnRH neurons. Another evidence for the cell migration-independent effect of PROK2-PROKR2 signalling on GnRH neurons is the fact that approximately 50% of Prok2-knockout mice have asymmetric olfactory bulbs [11]. In these mice, some GnRH neurons reach the hypothalamus but do not seem to adequately maintain the reproductive axis [36]. Although neuronal and functional connections between GnRH and other hypothalamic neurons are still unclear in humans, observations in rodent models and our patient collectively suggest that PROK2-PROKR2 signalling has a certain regulatory role on GnRH neurons after migration.

Our patient presented with two atypical features: obesity and intellectual disability. In rats, microinjection of prokineticin 2 into the arcuate nucleus decreased food intake, suggesting that Prok2-Prokr1 signalling is involved in the regulation of feeding behavior [37]. Indeed, some cases with heterozygous mutations showed obesity or metabolic syndrome [23]. However,
the relationship between biallelic PROKR2 variants and obesity in humans is not clear, as only two of the 15 carriers with reported BMI values were obese (BMI >30) (Table 1). Intellectual disability has never been documented in biallelic PROKR2 variant carriers. As for intelligence disability, we found no patient with this complication in the literature; thus, we presume it was unrelated to the PROKR2 variants.

In summary, we performed a comprehensive genetic analysis in a patient with CHH, and identified biallelic PROKR2 variants for the first time in Japan. The patient was characterized by a normal sense of smell and relatively high serum gonadotropin levels compared to previous cases. These clinical observations suggest that PROK2-PORKR2 signalling is involved not only in the migration of GnRH neurons but also in their functionality after migration, thereby providing a unique insight into the reproductive axis in humans.

Acknowledgments

We would like to thank Editage (www.editage.com) for English language editing. This study was supported by a grant from the Ministry of Health, Labour and Welfare, Japan (Jitsuyoka | Nanbyo| Ippan-014).

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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