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Additional mutation in *PROKR2* and phenotypic differences in a Kallmann syndrome/normosmic congenital hypogonadotropic hypogonadism family carrying *FGFR1* missense mutation

Kentaro Ichioka ,[1] Takeshi Yoshikawa,[2] Hiroko Kimura,[3] Ryoichi Saito[4]

[1]Karasumaoike Branch, Ichioka Urological Clinic, Kyoto, Japan
[2]Kyoto Station Branch, Ichioka Urological Clinic, Kyoto, Japan
[3]Mens Fertility Clinic Tokyo, Ichioka Urological Clinic Tokyo Branch, Tokyo, Japan
[4]Department of Urology, Kyoto University Graduate School of Medicine, Kyoto-shi, Japan

Correspondence to
Dr Kentaro Ichioka;
ichioka_kentaro@ybb.ne.jp

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SUMMARY

Congenital hypogonadotropic hypogonadism (CHH) is a genetically and clinically diverse disorder encompassing Kallmann syndrome (KS) and normosmic CHH (nCHH). Although mutations in numerous genes account for nearly 50% of CHH cases, a significant portion remains genetically uncharacterized. While most mutations follow the traditional Mendelian inheritance patterns, evidence suggests oligogenic interactions between CHH genes, acting as modifier genes to explain variable expressivity and incomplete penetrance associated with certain mutations.

In this study, the proband presented with nCHH, while his son exhibited KS. We employed whole-exome sequencing (WES) to investigate the genetic differences between the two, and Sanger sequencing was used to validate the results obtained from WES.

Genetic analysis revealed that both the proband and his son harboured a mutation in *FGFR1* gene. Notably, an additional rare mutation in *PROKR2* gene was exclusively identified in the son, which suggests the cause of the phenotypic difference between KS and nCHH.

BACKGROUND

Congenital hypogonadotropic hypogonadism (CHH) is a complex and clinically diverse disorder characterised by inadequate secretion or action of gonadotropin-releasing hormone (GnRH), resulting in deficient gonadal steroid production and impaired reproductive function. The clinical spectrum of CHH encompasses various conditions, including Kallmann syndrome (KS) and normosmic CHH (nCHH). KS is characterised by the association of CHH with anosmia or hyposmia, reflecting an underlying defect in both the hypothalamic–pituitary–gonadal (HPG) axis and olfactory system development. In contrast, nCHH refers to CHH without olfactory abnormalities.

Over the past decade, significant progress has been made in unravelling the genetic basis of CHH. More than 60 genes involved in the development and function of the HPG axis and olfactory bulb have been implicated, including *FGFR1* and *PROKR2*.[1] However, despite the identification of mutations in these genes, a substantial proportion of CHH cases remains genetically uncharacterised, suggesting the existence of additional genetic contributors.[2 3]

Intriguingly, CHH genetics exhibit substantial complexity, extending beyond the traditional Mendelian inheritance patterns. It has become increasingly evident that multiple genes can interact in an oligogenic manner, modulating the phenotypic expression and penetrance of specific mutations. These modifier genes play a crucial role in the severity and clinical presentation of CHH.[1]

In this study, we aimed to investigate the presence of modifier genes and their impact on phenotypic differences in CHH and KS. Specifically, we focused on a rare familial case in which the proband presented with nCHH while his son exhibited KS. We used whole-exome sequencing (WES) to identify potential genetic variants contributing to the observed phenotypic divergence.

CASE PRESENTATION

The proband is in his 40s and was diagnosed with nCHH at the age of 18 by the absence of puberty and results of hormone stimulating tests. He does not have any physical abnormality, nor olfactory dysfunction. MRI did not detect any abnormal finding in the pituitary. He undertook hormone replacement therapy with human chorionic gonadotropin and human menopausal gonadotropin or recombinant follicle-stimulating hormone and obtained normal spermatogenesis. During the disease management, he fathered one child.

The proband's son has anosmia, which was confirmed by Alinamin test, and lack of puberty at the age of 16. He has a history of surgery for cleft lip and palate. MRI did not detect any abnormal finding in the pituitary. With the results of hormone stimulating tests, he was diagnosed with KS.

The proband's wife is in her 40s, who is healthy but has a history of self-limited delayed puberty (SLDP). She did not have her first menstrual period until she was 18 but then spontaneously had one and was diagnosed with SLDP (figure 1).

INVESTIGATIONS

Methods

Whole-exome sequencing

We extracted genomic DNA from peripheral blood of the proband, his son and unaffected wife using QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). For WES, samples from the proband and his son were used. Exome library preparation was done with Agilent SureSelect Human All

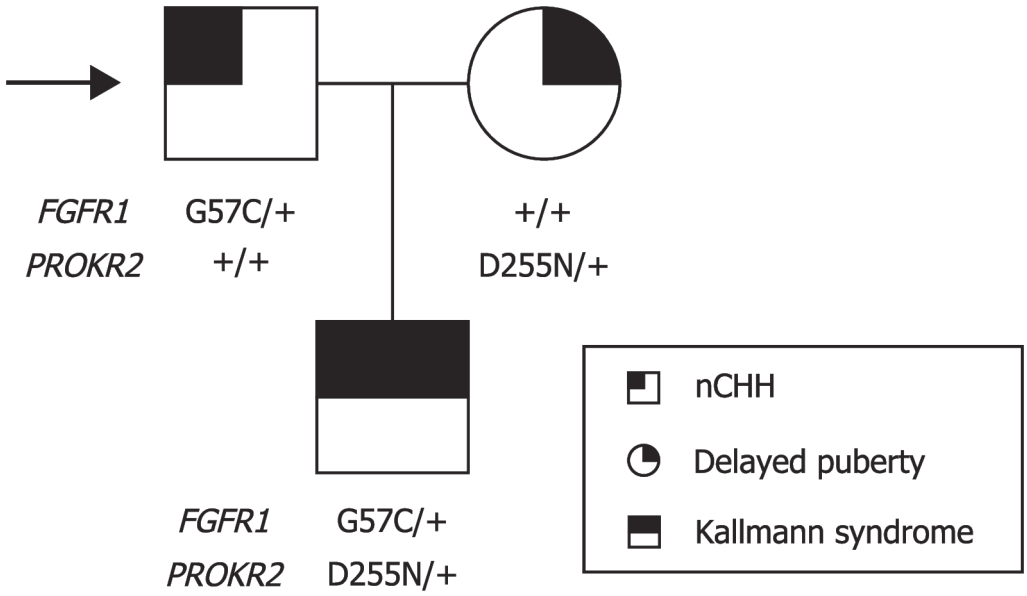

Figure 1 Pedigree of the patient. Arrows identify the normosmic congenital hypogonadotropic hypogonadism proband. Plus sign (+) indicates the wild-type allele. Squares represent males; circle represents female. Kentaro Ichioka created the figure.

Exon V6 kit (Agilent Technologies, Santa Clara, CA, USA), and sequencing was performed by Macrogen Japan using the NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Read processing, mapping to human genome reference (GRCh37-derived alignment set used in 1000 Genomes Project), variant calling, annotations and filtering for rare variants of minor allele frequency less than 0.01 affecting the coding sequence and/or consensus splice sites were also performed.

Sanger sequencing

The final results were confirmed with Sanger sequencing. Primers used were as follows: *FGFR1*-F GCA ATC CAC GCC TTG TCC TA and *FGFR1*-R GCT ATC CTG ACT CTG CCC CT: *PROKR2*-F ATG GCA GCC CAG AAT GGA AA and *PROKR2*-R TAG TCC ATC TCG AAG GGG CA.

In silico analysis

We checked our data with several in silico prediction model tools and data archives for pathogenicity. We used Sorting Intolerant from Tolerant (SIFT), likelihood ratio test (LRT), MutationTaster, MutationAssessor, Functional Analysis through Hidden Markov Models (FATHMM), Protein Variation Effect Analyzer (PROVEAN), Mendelian Clinically Applicable Pathogenicity (M-CAP), FATHMM-multiple kernel learning (FATHMM-MKL) and ClinVar. Regarding oligogenicity, we referred Oligogenic Disease Database (OLIDA) and used Oligogenic Resource for Variant Analysis (ORVAL), which can predict candidate pathogenic variant combinations in any gene pair and further predict their digenic effect.

Results

WES and Sanger sequencing

The proband and his son were found to be heterozygous for a *FGFR1* missense variant (c.763G>A; p.Asp255Asn). Notably, an additional heterozygous rare missense mutation in the *PROKR2* (c.169G>T; p.Gly57Cys) was exclusively identified in the son. Therefore, the proband's wife was examined and found to be harbouring the same heterozygous mutation in the *PROKR2* (figure 2).

No genetic mutations were found in the three of them for other CHH/KS-related genes.

In silico analysis

As for the *FGFR1* missense variant (c.763G>A; p.Asp255Asn), seven of eight prediction model tools indicate 'Damaging' or 'Disease-causing', while only FATHMM 'Tolerated'. There is no information in ClinVar. Regarding the *PROKR2* missense variant (c.169G>T; p.Gly57Cys), six of eight prediction model tools predict 'Damaging' or 'Deleterious', while SIFT and FATHMM 'Tolerated'. In ClinVar, the *PROKR2* mutation is classified as 'likely benign'. We could not retrieve any result from our data set with ORVAL. Referring to OLIDA, *FGFR1* and *PROKR2* have possible gene combination with monogenic+modifier pattern, although the variants shown are different from those of our cases.

OUTCOME AND FOLLOW-UP

The proband has been continuing hormone replacement therapy with testosterone enanthate ever since he finished procreation. Administration of 250 mg testosterone enanthate intramuscular injection every 3 weeks led to improvement of serum testosterone level. His son started hormone replacement therapy with human chorionic gonadotropin and recombinant follicle-stimulating hormone. Administration of 1500 U human chorionic gonadotropin and 150 U recombinant follicle-stimulating hormone every 2 days for 6 months induced normal puberty.

DISCUSSION

Our findings highlight that the presence of an additional mutation in *PROKR2* may contribute to the observed phenotypic differences between KS and nCHH in this family. These results underscore the significance of modifier genes and oligogenic inheritance in shaping the clinical manifestations of CHH.

PROKR2 expression is prominently observed in the arcuate nucleus, olfactory tract and suprachiasmatic nucleus, and is fundamental for migration and development of both GnRH and olfactory neurons.[4 5] The pioneering work of Dodé *et al* initially established the association of *PROKR2* with CHH.[6] In earlier

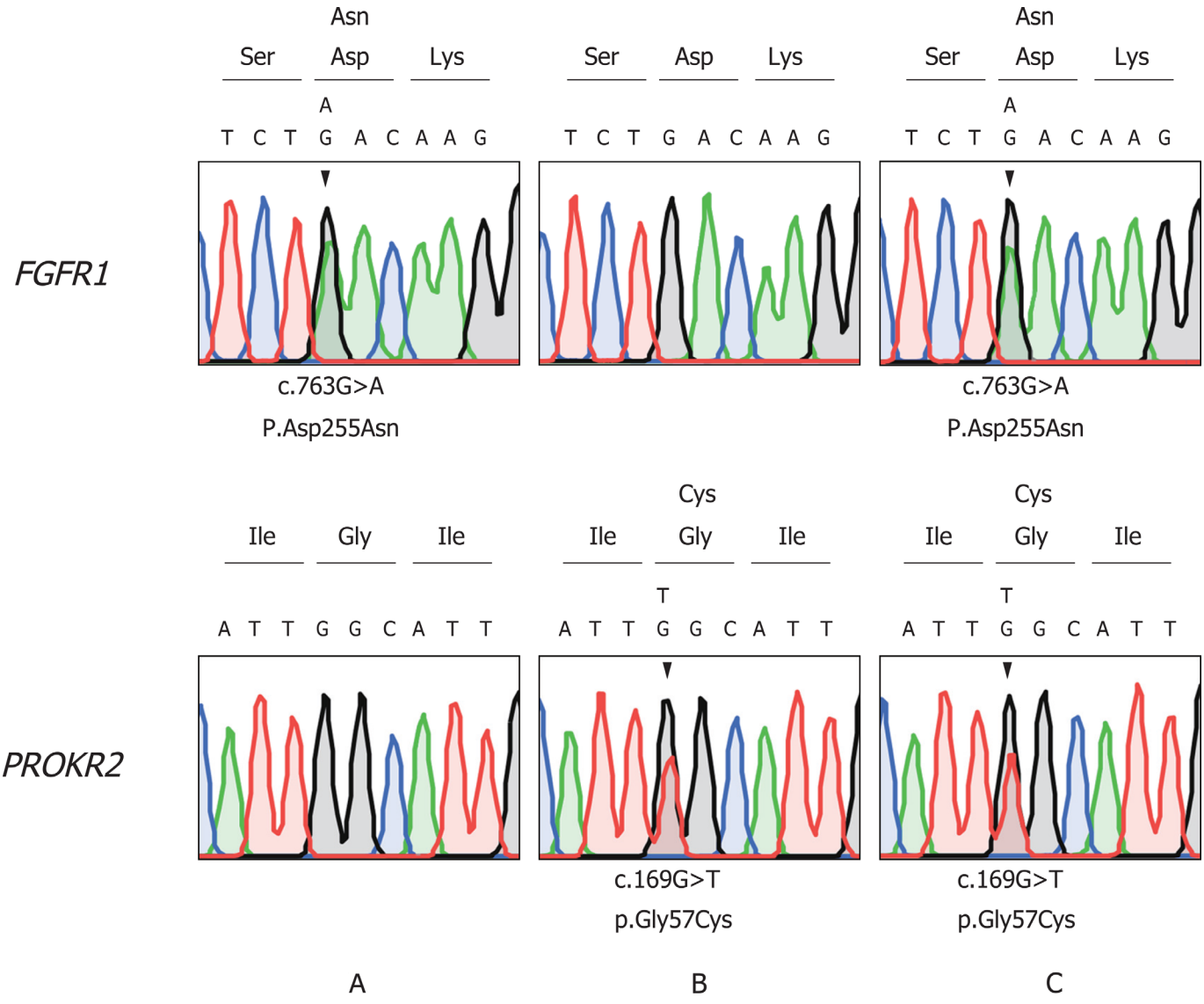

Figure 2 Partial electropherograms of PCR products amplified from *FGFR1* and *PROKR2* using genomic DNA of the congenital hypogonadotropic hypogonadism proband (A), the delayed puberty mother (B) and the Kallmann syndrome child (C). Kentaro Ichioka created the figure.

studies, a predominant proportion of *PROKR2* mutations were identified as missense variants.[1] Notably, patients with these mutations displayed an inconsistent phenotype, attributed to the variability in expressivity and penetrance. While homozygous mutations were consistently linked to a severe and highly penetrant phenotype,[6–9] the relationship between genotypes of *PROKR2* heterozygous variants and clinical manifestations remained elusive in the previous pedigree investigations.[6 10–15] Notably, certain functionally validated variants initially classified as exhibiting autosomal dominant inheritance with complete expressivity and penetrance were subsequently encountered in cases of adult-onset hypogonadotropic hypogonadism,[9 16] cases of reversal[17] and even among unaffected subjects.[7 18] Given the intricate nature of the heterozygous phenotype, which lacks a clear genotype–phenotype correlation, it appears that the influence of rare variants in other genes is often intertwined. Oligogenicity, the interaction of multiple genetic factors, may be essential for the pathogenicity of heterozygous *PROKR2* variants.

It is widely acknowledged that the timing of puberty in normal populations is heavily influenced by genetic factors.[19] Zhu *et al* conducted an investigation into the potential shared genetic basis between CHH and SLDP.[20] They used WES to analyse 15 families, each with a CHH-affected individual carrying a potentially harmful genetic variant in CHH-related genes. The study encompassed CHH probands and both family members with and without delayed puberty. Additionally, Cassatella *et al* explored the genetic architectures associated with CHH and SLDP and noted that potentially pathogenic variants in SLDP patients were found in *PROKR2* genes.[21]

FGFR1 plays a key role in the development of GnRH neurons' migration to the hypothalamus and the maturation of the olfactory bulb. Loss-of-function mutations in *FGFR1* can disrupt these processes, causing abnormal GnRH neuron migration and irregular olfactory bulb development. However, some individuals exhibit a typical nCHH phenotype without any olfactory dysfunction, which is highlighting the existence of *FGFR1*'s undefined role. *FGFR1* was the first gene where inactivating mutations were found in both nCHH and KS.[22] Roughly 10% of CHH patients have identified loss-of-function *FGFR1* mutations, primarily missense variants. While it could be inherited in an autosomal dominant manner, it often shows incomplete penetrance, interfamily variability and oligogenicity. Additionally,

affected individuals often show skeletal issues like craniofacial clefting, digit anomalies and dental agenesis.[23 24]

Through this analysis, we identified an additional rare mutation in *PROKR2* gene, which was exclusively present in the son and absent in the proband, while they are sharing the same mutation in *FGFR1* gene. Phenotypically, only the son has anosmia, cleft lip and palate. Although our study might be criticised for possibility of *FGFR1* monogenic disease model with coincidental presence of the identified *PROKR2* variant in the patient or a lack of functional analysis of these mutations in vitro and in silico,[25] the identification of this additional mutation in *PROKR2* raises intriguing questions regarding its role as a modifier gene and its potential contribution to the distinct phenotypic manifestations of KS and nCHH within the same family.

Patient's perspective

I am grateful for the diagnosis and initiation of treatment for my son. I suspected that my son might have the same disease as me, but I am glad that you correctly diagnosed and treated him. I am relieved that my son seems to be growing well.

Learning points

- Congenital hypogonadotropic hypogonadism (CHH) is a clinically diverse disorder that includes Kallmann syndrome (KS) and normosmic CHH (nCHH). More than 60 genes are linked to CHH and multiple genes can interact in an oligogenic manner and modifier genes can play a crucial role in the severity and clinical presentation of CHH.
- In the presenting family, the nCHH proband and KS son are sharing the mutation in *FGFR1* gene, while an additional rare mutation in *PROKR2* gene was exclusively present in the son and absent in the proband. The *PROKR2* mutation is derived from the proband's wife, who is healthy but has a history of self-limited delayed puberty (SLDP). CHH and SLDP can share the same genetic origin.
- Phenotypically, only the son has anosmia, cleft lip and palate. These phenotypic differences may be attributed to *PROKR2* mutation, which raise intriguing questions regarding its role as a modifier gene.

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Case reports provide a valuable learning resource for the scientific community and can indicate areas of interest for future research. They should not be used in isolation to guide treatment choices or public health policy.

ORCID iD

Kentaro Ichioka http://orcid.org/0000-0002-5395-0240

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