

Case Report

Combined pituitary hormone deficiency with unique pituitary dysplasia and morning glory syndrome related to a heterozygous *PROKR2* mutation

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Abstract. Recent reports have indicated the role of the prokineticin receptor 2 gene (*PROKR2*) in the etiology of congenital hypopituitarism, including septo-optic dysplasia and Kallmann syndrome. In the present study, using next-generation targeted sequencing, we identified a novel heterozygous *PROKR2* variant (c.742C>T; p.R248W) in a female patient who had combined pituitary hormone deficiency (CPHD), morning glory syndrome and a severely malformed pituitary gland. No other mutation was present in 27 genes related to hypogonadotropic hypogonadism, pituitary hormone deficiency and optic nerve malformation. The substituted amino acid was located on the third intracellular loop of the *PROKR2* protein, which is a G protein-coupled receptor. Computational analyses with two programs (SIFT and PolyPhen-2) showed that the substitution was deleterious to *PROKR2* function. The p.R248W mutation was transmitted from the patient's mother, who had a slightly delayed menarche. Collectively, we provide further genetic evidence linking heterozygous *PROKR2* mutations and the development of CPHD.

Key words: combined pituitary hormone deficiency (CPHD), morning glory syndrome, pituitary dysplasia, *PROK2*, *PROKR2*

Introduction

Loss-of-function mutations in *PROK2* and *PROKR2* (encoding prokineticin-2 and

prokineticin receptor-2, respectively) have been implicated in Kallmann syndrome (KS), which is characterized by hypogonadotropic hypogonadism (HH) and anosmia (1–3). In general, subjects with biallelic *PROKR2* mutations exhibit a fairly severe reproductive phenotype in both mice and humans (4, 5); however, the majority of *PROKR2* mutation-carrying patients harbor one heterozygous mutation and have variable expressivity of both the reproductive and olfactory phenotypes (1–5). Recently, *PROKR2* mutations were reported in patients with combined pituitary hormone deficiency (CPHD), including septo-optic

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Table 1 Hormonal evaluations of the proband at 11 yr of age

Provocation test with insulin-induced hypoglycemia, GnRH and TRH						
Min	0	15	30	60	90	120
BG (mg/dL)	70	41	48	74	83	81
GH (ng/mL)	3.27	1.08	0.6	4.11	3.81	1.91
LH (mIU/mL)	3.5	11.6	15.5	13.9	13.6	11.1
FSH (mIU/mL)	9.1	11.7	14.4	15.2	15.9	16.6
TSH (μ IU/mL)	0.90	5.85	5.55	4.38	3.63	2.80
PRL (ng/mL)	15.62	47.12	46.57	33.86	27.96	22.36
ACTH (pg/mL)	26.2	16.6	56.3	34.7	25.3	32.0
Cortisol (μ g/dL)	14.3	9.9	11.0	21.8	16.2	14.7
Provocation test with arginine						
Min	0	30	45	60	90	120
GH (ng/mL)	3.68	6.88	7.96	3.53	1.11	1.84
Basal hormonal data						
IGF-I (ng/mL)	120	FT ₃ (pg/mL)	4.27			
IGFBP-3 (ng/mL)	2.19	FT ₄ (ng/dL)	1.10			
E ₂ (pg/mL)	15.3					

BG, blood glucose; FT₃, free T₃; FT₄, free T₄; IGFBP-3, IGF binding protein-3.

dysplasia (SOD) (6–8), suggesting a potential role for the PROK2 pathway in pituitary development, in addition to its role in GnRH neuron development.

In the present study, we used next-generation targeted sequencing to screen for the genetic cause(s) of CPHD and identified a novel heterozygous *PROKR2* substitution (c.742C>T; p.R248W) in a patient with CPHD accompanied by marked pituitary dysplasia and morning glory syndrome.

Case Report

Clinical history

We report the case of a female Japanese patient who was 21 yr old at the commencement of our study. She was the second child born to unrelated healthy parents after a full-term pregnancy. Her mother attained menarche at the age of 14 yr, had regular menstrual cycles until 48 yr of age, and had not undergone treatment for infertility. At birth, the weight and height of the patient were 3110 g and 48 cm, respectively.

She required hospitalization for 2 weeks after delivery for respiratory distress caused by nasopharyngeal stenosis. At the age of 2 mo, she was diagnosed with morning glory syndrome in both eyes, resulting in amblyopia. Her early developmental milestones were reported to be within the normal range, but she did not walk until 18 mo of age. A dentist who treated the patient for misaligned teeth referred her to our Department of Endocrinology for an evaluation of short stature at the age of 11 yr. At this time, she was 122.4 cm tall (–3.3 SD) and weighed 24.6 kg, and she did not present with any other apparent anomalies.

Medical evaluation of the blood chemistry of the patient showed normal results. The results of endocrine provocation tests are shown in Table 1. The GH responses to insulin-induced hypoglycemia and arginine were low. The serum IGF-I level was low (129 ng/mL; –3.24SD; age-specific reference) (9). The responses of gonadotropins to GnRH stimulation were prolonged, although the peak gonadotropin levels were appropriate for her pubertal stage (Table

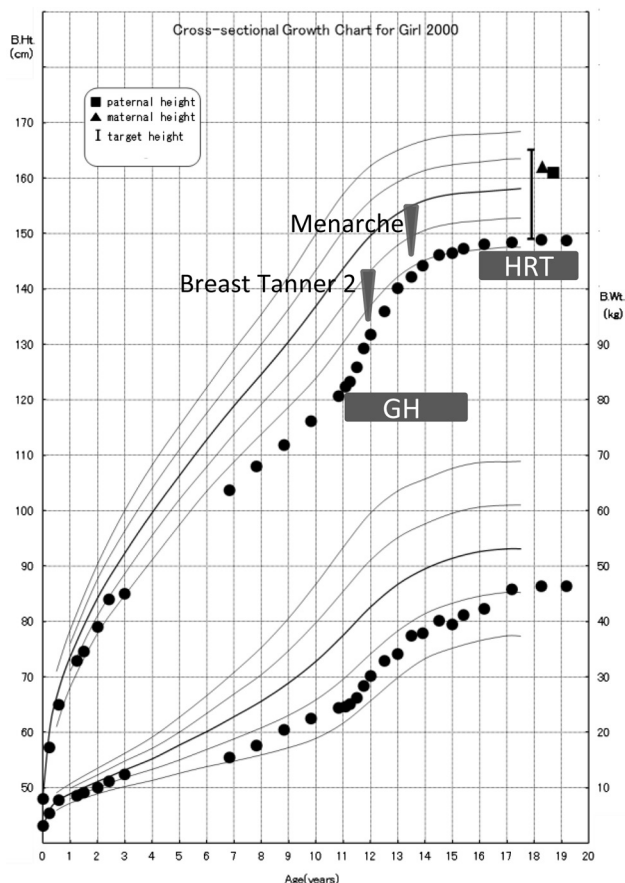


Fig. 1. Growth chart of the patient. Recombinant GH at a physiological dose induced a remarkable change in her growth rate. Breast development occurred spontaneously at the age of 11 yr and 11 mo, and menarche occurred at the age of 13 yr and 6 mo, but she had never had regular menstrual cycles; hormone replacement therapy was started due to amenorrhea.

1). The patient's thyroid axis and adrenal axis were intact. Recombinant GH at a physiological dose induced a remarkable change in her growth rate (Fig. 1). Breast development occurred spontaneously at the age of 11 yr and 11 mo, and menarche occurred at the age of 13 yr and 6 mo, but she had never had regular menstrual cycles. By the age of 15 yr, she had developed amenorrhea, and hormone replacement therapy was started. Her adult height was improved by supplementation with recombinant GH, and she reached a height of 148.8 cm (-1.8 SD).



Fig. 2. A, Midsagittal reconstruction in plane computed tomography. The bony floor of the sella was defective (white arrowhead), and a downward extension of the tumorous structure through the defect (white arrow) was identified. B and C, T1-weighted three-dimensional images (1 mm thickness) B, reconstructed coronal image showing duplicated pituitary stalks (black arrows) C, pituitary gland identified in the deformed sella. The posterior lobe showed a region of high intensity (black arrowhead). D, Midsagittal spin echo image (2 mm thickness) revealed a tumorous structure below the sella turcica that extended to the nasopharynx through the defective sella floor; the fat signal intensity indicates a dermoid tumor or teratoma.

Computed tomography and magnetic resonance imaging

Computed tomography revealed a bony defect in the floor of the sella turcica and a tumorous soft tissue structure that extended downward through the defect (Fig. 2A). Magnetic resonance imaging of the hypothalamo-pituitary region with a 1.5-T scanner showed marked dysmorphic structures. A duplicated pituitary stalk with the right stalk continuing to the pituitary gland in the deformed sella was found

(Fig. 2, B and C). A tumorous structure extending to the nasopharynx was also detected (Fig. 2D); a dermoid tumor or a teratoma was suspected based on its fat signal intensity. The olfactory sulci and bulbs were normal (data not shown).

Molecular studies

We obtained written informed consent from the patient and her parents for molecular studies. This study complied with the Helsinki Declaration of 1975, revised in 1983, and was approved by the institutional review board of Kanagawa Children's Medical Center. Leukocytic DNA was isolated from the patient and her parents with standard techniques. Array comparative genomic hybridization analysis showed no significant genomic rearrangement. Direct sequencing of 10 genes implicated in congenital hypopituitarism (*POU1F1*, *PROP1*, *LHX3*, *LHX4*, *SOX2*, *SOX3*, *HESX1*, *PAX6*, *SIX6*, and *GLI2*) revealed no mutation. We then performed next-generation targeted sequencing for HH and hypopituitarism. We tested 25 genes, including *KAL1*, *FGFR1*, *FGF8*, *GNRH1*, *GNRHR*, *KISS1*, *KISS1R*, *LHB*, *FSHB*, *CHD7*, *PROK2*, *PROKR2*, *TAC3*, *TACR3*, *HESX1*, *GLI2*, *LHX4*, *OTX2*, *PAX6*, *POU1F1*, *PROP1*, *SOX2*, *GH1*, *GHRH* and *GHRHR* using a MiSeq instrument (Illumina Inc., San Diego, CA, USA) according to the SureSelect protocol (Agilent Technologies, Santa Clara, CA, USA), as previously described (10) with minor modifications. As a result, we found a novel heterozygous *PROKR2* substitution (c.742C>T, p.R248W) (Fig. 3). This substitution was not present in 150 control alleles and has not been reported in the Single Nucleotide Polymorphism Database (dbSNP) or the Human Genetic Variation Database (HGVD). The substituted amino acid was located on the third intracellular loop of the *PROKR2* protein (Fig. 4). The functional effects of the substitution were analyzed using SIFT and PolyPhen-2. These tools indicated that the substitution was deleterious (score of 0.02) and probably damaging (score of 0.966), respectively. No other mutation was identified

among the genes analyzed in this study. Family analysis revealed that the *PROKR2* p.R248W mutation was transmitted from the mother.

Discussion

The p.R248W mutation of the *PROKR2* gene identified in our patient was not observed in ethnicity-matched controls or any variation/mutation database, indicating that this substitution is extremely rare. The affected residue (Arg248) is evolutionarily conserved, and p.R248W was judged to be a deleterious variant by the two most popular programs for functional effect prediction (SIFT and PolyPhen-2). A different substitution of the same amino acid, R248Q (c.743G>A), has been reported in a patient with a mild form of HH (Fig. 4), and functional analysis of the p.R248Q mutant revealed a mild decrease in calcium influx (3). This case report suggests that R248 is important for the function of *PROKR2*, and supports the hypothesis that the heterozygous p.R248W substitution identified in the present study is deleterious to protein function, though expression experiments will be required for a more rigorous evaluation of its pathogenicity.

The *PROK2* pathway was initially studied for its involvement in gastrointestinal smooth muscle contraction, angiogenesis, hematopoiesis and circadian rhythms. The knockout models for both *Prok2* and *Prokr2* revealed unexpected roles of the pathway in olfactory bulb morphogenesis and sexual maturation (11, 12). In fact, sequencing of *PROK2* and *PROKR2* among patients with HH showed that a subset of patients had mutations in both genes (1–5, 13). More recently, an association between *PROK2* pathway defects and the development of CPHD with or without SOD has been discussed (6–8). Reynaud *et al.* (7) screened for the *PROK2* and *PROKR2* genes among 72 index cases with pituitary stalk interruption syndrome from the GENHYPOPIT database and found three heterozygous *PROKR2* variant carriers. These reports suggest a potential

inheritance is currently considered to be the most plausible explanation for the phenotypes observed in patients with heterozygous mutations. We also observed a considerable phenotypic difference between the two mutation carriers in the present study (the patient and her mother), but no mutation to synergize was found other than *PROKR2*. Further investigations will be required to clarify the phenotypic variability among patients with only one heterozygous *PROKR2* mutation. Nevertheless, our findings suggest that *PROKR2* should be evaluated as a candidate responsible gene in patients with CPHD resulting from pituitary dysplasia in addition to KS patients.

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