ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2023.2023-4-4

J Clin Res Pediatr Endocrinol 2023;15(4):338-347

PROKR2 Mutations in Patients with Short Stature Who Have Isolated Growth Hormone Deficiency and Multiple Pituitary Hormone Deficiency

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What is already known on this topic?

Homozygous *PROKR2* mutations have been identifed in Kallmann syndrome and hypogonadotropic hypogonadism. Recently, PROKR2 has been suggested to play a role in pituitary hormone deficiencies. While homozygous *PROKR2* mutations have been reported as pathogenic, the role of heterozygous forms in the mechanism is unknown.

What this study adds?

This study presents strong evidence that heterozygous *PROKR2* mutations play a role in pituitary hormone deficiencies other than Kallmann syndrome. Heterozygous healthy carriers suggest that concomitant oligogenic or digenic inheritance in patients with *PROKR2* mutation is the strongest underlying mechanism of disease causing phenotype.

Abstract

Objective: Recent reports have indicated the role of the prokineticin receptor 2 gene *(PROKR2)* in the etiology of pituitary hormone deficiencies, suggesting a potential role for the PROK2 pathway in pituitary development, in addition to its role in gonadotropin releasing hormone-expressing neuron development. Here, we present the clinical and molecular findings of four patients with *PROKR2* mutations.
Methods: Next-generation targeted sequencing was used to screen 25 genes in 59 unrelated patients with multiple pituitary hormone deficiency (MPHD), isolated growth hormone (GH) deficiency, or idiopathic short stature.
Results: Two different, very rare *PROKR2* missense alterations classified as pathogenic (NM_144773.4:c.518T>G; NP_658986.1:p.(Leu173Arg)) and likely pathogenic (NM_144773.4:c.254G>A; NP_658986.1:p.(Arg85His)) were identified in four patients in heterozygous form. Patient 1 and Patient 2 presented with short stature and were diagnosed as GH deficiency. Patient 3 and Patient 4 presented with central hypothyroidism and cryptorchidism and were diagnosed as MPHD. No other pathogenic alterations were detected in the remaining 24 genes related to short stature, MPHD, and hypogonadotropic hypogonadism. Segregation analysis revealed asymptomatic or mildly affected carriers in the families.
Conclusion: *PROKR2* dominance should be kept in mind as a very rare cause of GH deficiency and MPHD. Expressional variation or lack of penetrance may imply oligogenic inheritance or other environmental modifiers in individuals who are heterozygous carriers.
Keywords: Growth hormone deficiency, multiple pituitary hormone deficiency, *PROKR2*, short stature

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Conflict of interest: None declared
Received: 09.05.2023
Accepted: 19.06.2023

Introduction

The prokineticin system consists of two multifunctional proteins, prokineticin-1 and prokineticin-2, and their G protein-coupled receptors. They were first identified in 2000 by Li et al. (1) as endogenous regulators of the gastrointestinal tract. More recently it has been shown that they have roles in many biological functions, such as circadian rhythm regulation, nociception, angiogenesis, hematopoiesis, immune response, development of the olfactory bulb, and sexual maturation. Expression of prokineticins and their receptors has been reported in various tissues, including the ovary, testis, uterus, adrenal gland, placenta, brain, digestive tract, heart, and bone marrow (2,3,4). As the prokineticin signaling pathway has a critical role in the embryonic development of the olfactory system, it was proposed that both neural and neuroendocrine developmental abnormalities could occur in patients carrying mutations in these genes (5). In *PROK2* and *PROKR2* knockout mice, gonadotropin-releasing hormone (GnRH) secretion was impaired which led to a disruption of sexual development and fertility in both male and female mice, thus making the *PROK2* and *PROKR2* genes strong candidates for human GnRH deficiency (6,7,8).

In recent years the number of patients with Kallmann syndrome who have *PROKR2* mutation has increased (9,10,11). In addition, monoallelic *PROKR2* variants were reported to have a role in multiple pituitary hormone deficiency (MPHD) and septooptic dysplasia (SOD) (12).

Despite this, healthy subjects were reported to have these same variants in heterozygous form (11,13). Therefore, it was proposed that these mutations did not cause major midline defects spontaneously but may contribute as modifier genes or induce the phenotype through digenic or oligogenic inheritance, as previously demonstrated in idiopathic hypogonadotropic hypogonadism (IHH) and Kallmann syndrome (7,14). Thus, further studies are needed to clarify the role of PROKR2 signaling in the pituitary gland and midline development (12).

In this study, a gene panel was used to screen for the genetic causes of MPHD, growth hormone (GH) deficiency, and idiopathic short stature. We identified four patients with *PROKR2* variants with different phenotypes other than Kallmann syndrome. The role of the PROKR2 gene in the etiology of GH deficiency and MPHD was investigated.

Methods

Patients

Using a candidate gene approach, 59 patients with MPHD, GH deficiency, and idiopathic short stature were screened. Written informed consent was obtained from all patients. The study protocol was approved by the İstanbul University, İstanbul Faculty of Medicine Local Clinical Research Ethics Committee (date: 11.08.2017, approval number: 13).

The data, collected retrospectively, consisted of physical examination, auxological findings, family history, hormone assays, biochemical and radiological findings, surgical and medical treatment, and additional features at follow-up (see below). Anthropometric measurements of the patients and parental heights were measured by the same auxologist and the target height was calculated. Bone age was evaluated by using the Greulich-Pyle method (15). The predicted adult height was calculated according to the Bayley Pinneau method (16). The standard deviation score (SDS) of all auxological measurements was calculated according to national data (17,18). The upper limit for Turkish girls to attain menarche is 14 years old and menarche after 14 years of age was defined as delayed menarche (19).

Molecular Analysis

Chromosomal abnormalities were excluded by using microarray and cytogenetic techniques before the initiation of molecular genetic analysis. Screening of targeted regions for an in-house-designed panel with 25 genes (*BMP4, FGF8, FGFR1, GH1, GHR, GHRH, GHSR, HESX1, HHIP, IGF1, IGF1R, IGFALS, IGFBP3, IGSF1, LHX3, LHX4, OTX2, POU1F1, PROKR2, PROP1, SHH, SHOX, SOX3, STAT5B, WDR11*) were tested using Ion Torrent PGM™ system for next-generation sequencing (ThermoFisher Scientific, Waltham, MA, USA).

Hormonal Assays

Blood samples were collected in the morning after eight hours of fasting. Luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, cortisol, free thyroxine, and thyroid stimulating hormone were analyzed by electrochemiluminescence immunoassay (Cobas, Roche Diagnostics, Mannheim, Germany). Insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein 3 (IGFBP-3) levels were analyzed by immunoradiometric assay (Immunotech, Beckman Coulter Inc, Prague, Czech Republic). GH was determined by radioimmunoassay (Diagnostic System Laboratories Inc., Webster, TX, USA). GH stimulation tests (GHST) were performed with clonidine and L-dopa and GH values less than 10 ng/mL were accepted as GH deficiency (20). GnRH test was performed and serum LH and FSH concentrations were measured at baseline and at 30, 60, 90, and 120 minutes after an intravenous bolus of 0.1 mg gonadorelin acetate. Bone mineral density L1-L4 was evaluated using dual-energy X-ray absorptiometry (Hologic QDR 4500A Fan Beam X-ray Bone Densitometer, Hologic, Bedford, MA, USA) and analyzed using software version 12.3.

Statistical Analysis

The Statistical Package for Social Sciences for Windows 21.0 was used for statistical analysis (IBM Inc., Armonk, NY, USA). Results are reported as median (minimum-maximum) or as number or percentages, where appropriate.

Results

General Results

Genetic analyses revealed two different heterozygous clinical variants in the *PROKR2* gene in four patients. These variants had previously been reported in Kallmann syndrome. Patient 1 and Patient 2 were heterozygous for NM_144773.4:c.254G > A;p.(Arg85His) and Patient 3 and Patient 4 were heterozygous for NM_144773.4:c.518T > G;p.(Leu173Arg) variants. Segregation in families revealed that the mothers of Patients 2 and 3, and the father of Patients 1 and 4 were the carriers of the related variants. Delayed puberty or short stature of carrier parents of three patients were associated with *PROKR2* mutation. However, we could not evaluate the hormone axes because the family members did not consent so that only hypothyroidism and hypogonadism were excluded. The father of Patient 4 could not be evaluated. Pedigrees are shown in Figure 1.

Patient 1

A 12-year-old female patient was referred for short stature. She was born into a consanguineous family at term with low birth weight and had no problems during the prenatal or early postnatal period. Her motor and mental developmental milestones were normal for her age. Family history revealed short stature in her father and delayed menarcheal age in her mother. Physical examination at presentation was normal, except for proportionate short stature. She had a normal sense of smell and no dysmorphic features.

Workup for short stature yielded normal biochemical investigations, thyroid hormone, cortisol, and prolactin levels. IGF-1 and IGFBP-3 levels were in normal ranges but GHSTs were compatible with GH deficiency. Cranial and pituitary magnetic resonance images (MRI) did not reveal any pathology. At 12.75 years of age, growth velocity decreased and GH treatment was started (0.035 mg/kg/day). She was treated with GH until the age of 13.9 years.

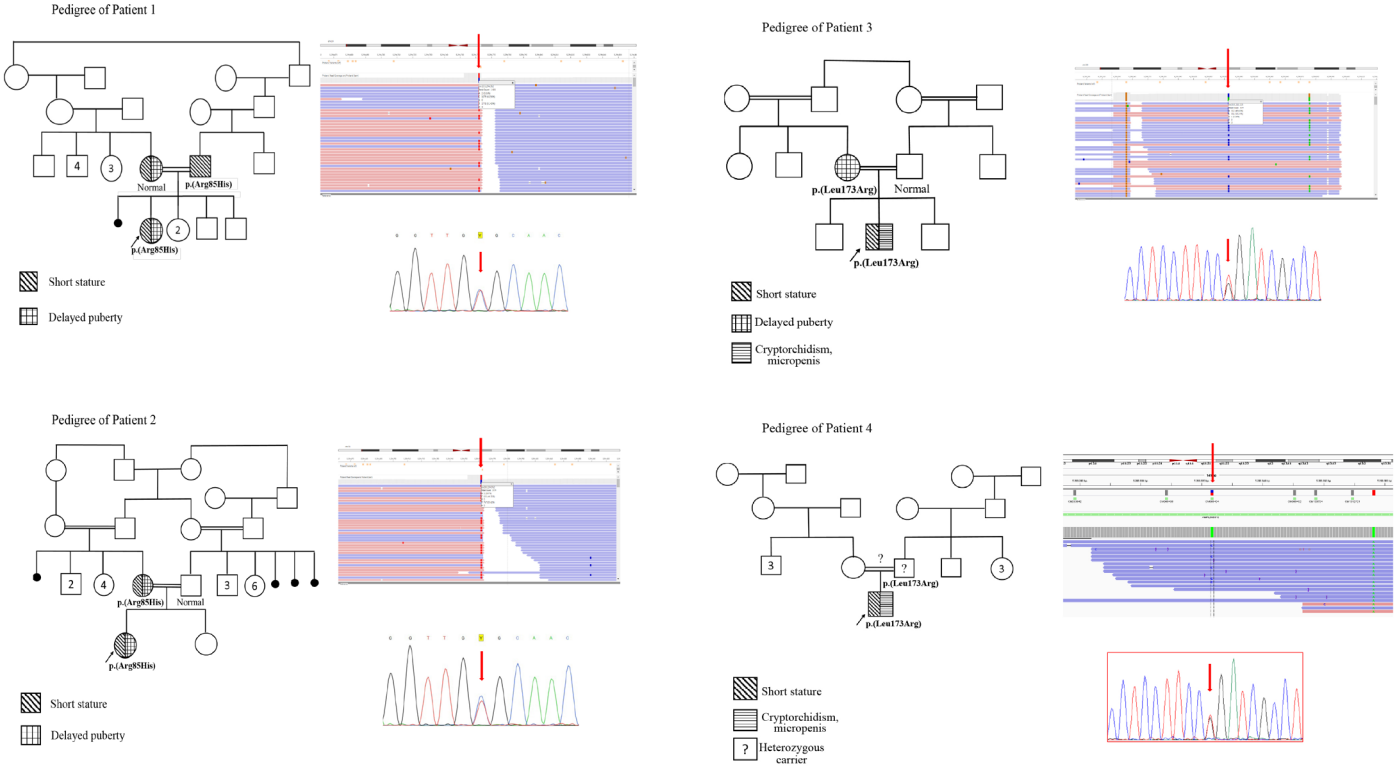

Figure 1. Family pedigrees of the patients with *PROKR2* allelic variants. Arrow points out the probands. The clinical signs that the symbols represent are given by line pattern. The Integrative Genomics Viewer of the variants and the electropherogram of the Sanger sequences of the variant sites are shown

At presentation, the patient was at Tanner stage 2 and menarche occurred at 13.5 years of age. At the last evaluation, the patient was 15.7 years old, pubertal development was complete and she had regular menstruation. GnRH test was performed and basal FSH and LH were 4.47 IU/L and 10.52 IU/L and increased to 10.52 IU/L and 31.77 IU/L, respectively. Urinary tract ultrasonography (USG) and pelvic USG were normal. The clinical and hormonal findings and the molecular results of the patients are shown in Table 1 and Table 2.

Table 1. Clinical and anthropometric findings in the patients

At presentation	Patient 1	Patient 2	Patient 3	Patient 4	Median (min-max)
Age (years)	12	11	0.5	0.5	5.75 (0.5-12)
Gender	F	F	M	M	-
Consanguinity	3rd degree	1st degree	No	3rd degree	-
Presenting features	Short stature	Short stature	Short stature, micropenis, undescended testis	Short stature, micropenis, undescended testis	-
Birth weight g/SDS	2500/-2.0	3600/0.7	3230/-0.6	2100/-2.6	-1.3 (-2.6-0.7)
Height cm/SDS	135/-2.7	128.4/-2.5	59.2/-3.3	51.1/-6.3	-3.0 (-6.3 and -2.5)
Weight kg/SDS	30.9/-2.0	31.4/-1.0	8.3/0.05	4.0/-4.8	-1.5 (-4.8-0.05)
BMI kg/m²/SDS	17/-0.8	19/0.4	23.5/3.3	15.0/-1.7	-0.2 (-1.7-3.3)
HC cm/SDS	52.8/-0.8	51.6/-1.2	43.2/-0.7	35.5/-6.1	-1.0 (-6.1 and -0.7)
SHR/SDS	0.54/0.9	0.53/-0.03	0.69	-	0.69 (-0.03-0.9)
Tanner stage	Ph1B2/2	Ph1B2/2	Ph1T0.5/0.5 mL	Ph1T nonpalpable	-
Bone age (years)	$8^{10/12}$-10	$7^{10/12}$-$8^{10/12}$	1 (at age of 1.6 years)	NA	-
Mother's height SDS	150.6/-1.9	147.4/-2.4	157.5/-0.9	156.5/-1.1	-1.5 (-2.4 and -0.9)
Father's height SDS	158.8/-2.4	167.2/-1.3	168.8/-1.1	NA	-1.3 (-2.4 and -1.1)
Target height cm/SDS	148.2/-2.3	150.8/-1.9	169.7/-0.9	NA	-1.9 (-2.3 and -0.9)
Hormone deficiencies (onset of age-years)	GH (12.3 years)	GH (11.2 years)	GH (14 months) TSH (6 months) PRL (10 months)	GH (22 months) TSH (6 months) PRL (6 months) FSH/LH (6 months) DI (6 months)	-
At most recent evaluation					
Age (years)	15.7	18.4	14.4	1.6	15.1 (1.6-18.4)
Height cm/SDS	146.5/-2.7	153.2/-1.7	173.2/0.8	65/-5.0	-2.2 (-5.0-0.8)
Weight kg/SDS	54.2/-0.3	57.3/-0.1	78/1.6	5.7/-6.1	-0.2 (-6.1-1.6)
SHR/SDS	0.54/0.1	0.52/0.1	0.54/1.4	NA	0.1 (0.1-1.4)
BMI SDS	1.4	1.1	1.4	-2.8	1.25 (-2.8-1.4)
Pubertal stage (Tanner stage)	Ph5B5/5	Ph5B5/5	Ph3T5/5 mL	Ph1T nonpalpabl	-
Bone age (years)	15	16	15	NA	-
Mother's menarcheal age (years)	16	15	14	14	14.5 (14-16)
Menarcheal age (years)	13.5	15.6	-	-	-
Replacement treatment (duration)	GH (until age 13.9)	GH (until age 17)	GH (until age 14.1) L-thyroxine (continue)	GH (started) L-thyroxine (continue) Desmopressin (continue)	-
Zygosity **NM_144773.2** **NP_658986.1** **HGMD id** **dbSNP id**	Heterozygous c.254G > A p.(Arg85His) CM065401 rs74315418	Heterozygous c.254G > A p.(Arg85His) CM065401 rs74315418	Heterozygous c.518T > G p.(Leu173Arg) CM065404 rs74315416	Heterozygous c.518T > G p.(Leu173Arg) CM065404 rs74315416	-
Parental carrier status	Father (+) Mother (-)	Father (-) Mother (+)	Father (-) Mother (+)	Father (+) Mother (-)	-

The median (min-max) of the anthropometric data with the standard deviation score was calculated.
F: female, M: male, SDS: standard deviation score, BMI: body mass index, HC: head circumference, SHR: sitting height ratio, Ph: pubic hair, B: breast, T: testis, NA: not available, GH: growth hormone, TSH: thyroid stimulating hormone, PRL: prolactin, DI: diabetes insipitus, min-max: minimum-maximum

Table 2. Laboratory and radiological results of the patients

	Patient 1	Patient 2	Patient 3	Patient 4	Normal ranges
Na (mmol/L)	139	139	141	147	135-145
K (mmol/L)	4.2	4.8	4.0	5.0	3.5-5.5
Cortisol (nmol/L)	496.8	574	472	265	77.3-635
fT4 (pmol/L)	15.8	16.9	8.3	8.6	11.6-21.5
TSH (mIU/L)	4.6	1.83	0.56	2.39	0.66-4.14
Prolactin (ng/mL)	6.9	19.4	1.9	0.7	4.8-23.3
IGF-1 (ng/mL) (Normal range)	259 (76-542)	67.8 (111-551)	<25 (55-237)	<25 (55-237)	-
IGFBP-3 (ng/mL) (Normal range)	4.67 (2.4-8.4)	2.53 (2.4-8.4)	0.502 (0.7-3.6)	<0.5 (0.7-3.6)	-
FSH (IU/L)	1.7	2.4	0.44	2.3	1.7-7.7
LH (IU/L)	0.6	0.5	0.1	0.2	1-11.4
Estradiol (pg/mL)	5	20.1	-	-	10-100
GHST peak GH (mg/L) Clonidine L-dopa	 1.7 0.14	 8.17 1.9	 0.105 0.08	 0.08 0.31	
MRI	Normal	Anterior pituitary hypoplasia	Normal	Pituitary hypoplasia, diffuse hypomyelination, cerebral atrophy	
Cortisol (nmol/L)	441.6	223.6	447.1	375.4	82.8-579.6
fT4 (pmol/L)	17.2	16.9	14.2	24.1	11.6-21.5
TSH (mIU/L)	3.26	2	0.007	0.005	0.66-4.14
Prolactin (ng/mL)	30.5	19.1	0.49	0.18	4.8-23.3
IGF-1 (ng/mL) (Normal range)	256 (191-496)	80.3 (117-323)	43.3 (120-501)	17.8 (41-225)	
IGFBP-3 (ng/mL) (Normal range)	5.22 (3.3-10)	2.22 (2.9-7.3)	2.47 (3.5-10)	416 (1410-2970)	
FSH (IU/L)	4.5	5.9	8.8	NA	1.7-7.7
LH (IU/L)	2.9	7.4	4.3	NA	1-11.4
Estradiol (pg/mL)	53.7	69.8	16.4	-	10-100
Testosterone (ng/mL)	-	-	2.1** (Tanner 2)	0.02* (Tanner 1)	* <0.02 **0.02-0.58
Bone density Z score BMD g/m^2	-0.1 0.812	-2.3 0.770	1.7 0.903	NA	>-1.0

NA: not available, Na: sodium, K: potassium, fT4: free thyroxine, TSH: thyroid stimulating hormone, IGF-1: insulin-like growth factor-1, IGFBP-3: insulin-like growth factor binding protein 3, FSH: follicle-stimulating hormone, LH: luteinizing hormone, GH: growth hormone, GHST: GH stimulation test, BMD: bone mineral density, MRI: magnetic resonance imaging

Patient 2

Patient 2 was referred for short stature at 11 years of age. She was born into a consanguineous family at term with normal birth weight and had no problems during the prenatal or early postnatal period. Her motor and mental developmental milestones were normal for her age. Family history revealed short stature and delayed menarche in her mother. At presentation, her physical examination was normal except for her short stature. Body proportions were normal. She had a normal sense of smell and no dysmorphic features.

Hormonal evaluation of the pituitary axis yielded normal results for prolactin, thyroid, and adrenal function. IGF-1 level was low and GH deficiency was diagnosed on GHSTs. Cranial and pituitary MRI revealed anterior pituitary gland hypoplasia.

At follow up mild gastrointestinal symptoms started and the patient's weight decreased to -3.0 SDS. A celiac diseaase work-up was negative and the patient was diagnosed with chronic duodenitis. After the correction of malnutrition, growth velocity remained low, therefore GH treatment was started (0.035 mg/kg/day) at the age of 15 years and continued until the age of 17 years.

At presentation, puberty was at Tanner stage 2. Basal serum LH and FSH concentrations were 1.6 IU/L and 2.2 IU/L and increased normally in response to GnRH stimulation (LH increased to 14.2 IU/L and FSH increased to 7.1 IU/L). Spontaneous menarche occurred at the age of 15.6 years

Table 3. The patients with MPHD and isolated GH deficiency reported to have heterozygous *PROKR2* variants

Patient	Ref	Hormone deficiency	Pituitary MRI	Phenotype	*PROKR2* gene NP_658986.1	Additional gene
1	12	GH, TSH, ACTH, LH, FSH	EPP, pituitary stalk agenesis	SOD, MPHD	p.(Arg268Cys)	
2	12	GH, TSH, LH, FSH	Normal	SOD, MPHD	p.(Arg85Gly)	
3	12	GH, TSH, ACTH, LH, FSH	NA	MPHD	p.(Arg85His)	*ANOS1* *NM_000216.4: c.1375C > T p.(His459Tyr)*
4	28	GH, TSH, ACTH, LH, FSH	APH, EPP, absent stalk, thin corpus callosum	PSIS	p.(Leu173Arg)	
5	28	GH, TSH, ACTH, LH, FSH	APH, EPP, interrupted pituitary stalk	PSIS	p.(Arg85His)	*HESX1* *NM_003865.3: c.200G > C p.(Ser67Thr)*
6	28	GH, TSH, ACTH, LH, FSH	EPP, interrupted pituitary stalk, porencephaly	PSIS	p.(Ala51Thr)	
7	28	GH	APH, thin pituitary stalk	Isolated GHD	p.(Ala51Thr)	
8	29	TSH, ACTH, GH	APH, EPP, thin interrupted stalk	PSIS	p.(Arg85Cys)	*WDR11* *NM_018117.12: c.1306A > G; p.(Ile436Val)*
9	30	GH, TSH, ACTH, LH, FSH	Absent anterior pituitary, EPP	MPHD	p.(Arg85Leu)	
10	30	GH, ACTH, TSH, DI	APH, partially descended PP	MPHD	p.(Leu173Arg)	
11	30	GH, ACTH, TSH, DI	Absent septum pellucidum	SOD, MPHD	p.(Leu173Arg)	
12	30	GH, ACTH, TSH	APH, EPP, hypoplastic stalk	MPHD	p.(Leu173Arg)	
13	30	GH, ACTH, TSH	APH, EPP	SOD, MPHD	p.(Leu173Arg)	
14	30	GH	APH	SOD	p.(Ala51Thr)	
15	30	GH, TSH, ACTH, LH, FSH	APH, EPP, hypoplastic stalk	SOD, MPHD	p.(Arg268Cys)	
16	30	GH, TSH, ACTH, LH, FSH	EPP, absent infundibulum	SOD, MPHD	p.(Arg268Cys)	
17	30	GH, TSH	Corpus callosum agenesis	SOD, MPHD	p.(Arg268Cys)	
18	30	GH	APH	SOD	p.(Gly371Arg)	
19	31	GH, ACTH, LH, FSH, DI	Absent posterior pituitary, absent stalk	MPHD	p.(Arg85Cys)	
20	31	GH, TSH, ACTH, LH, FSH	APH, EPP, absent stalk	MPHD	p.(Arg248Glu)	
21	32	ACTH, TSH	Normal	MPHD	p.(Leu173Arg)	
22	32	NA	NA	Hypopituitarism	p.(Arg85Cys)	
23	32	NA	NA	Hypopituitarism	p.(Arg85His)	
24	33	GH, ACTH, TSH, LH, FSH	APH, EPP, absent stalk, optic chiasm asymmetry	MPHD	p.(Glu231Lys)	TGIF1 NM_170695.4: c.90G > A; (p.Trp30Ter)
25	34	GH	Small anterior pituitary	Isolated GHD	p.(Pro12fs*30)	
26	35	GH	NA	Isolated GHD	p.(Trp178Ser)	
27	35	GH	NA	Isolated GHD	p.(Trp178Ser)	
28	35	GH	NA	Isolated GHD	p.(Trp178Ser)	
29	36	GH, LH, FSH	Duplicated pituitary stalk	MPHD, MGS	p.(Arg248Trp)	

GH: growth hormone, TSH: thyroid stimulation hormone, ACTH: adrenocorticotropic hormone, LH: luteinizing hormone, FSH: follicle stimulating hormone, MPHD: multiple pituitary hormone deficiency, PSIS: pituitary stalk interruption syndrome, SOD: septooptic displasia, APH: anterior pituitary hypoplasia, EPP: ectopic posterior pituitary, DI: diabetes insipidus, MGS: morning glory syndrome, NA: not available, GHD: growth hormone deficiency, MRI: magnetic resonance imaging

and the pelvic USG of the patient was normal. At the last evaluation, the patient was 18.4 years old, her height SDS was normal and she had regular menstruation.

Patient 3

Patient 3 was a 0.5-year-old male patient who was referred to the pediatric endocrinology clinic because of central hypothyroidism which was detected during the evaluation of poor height gain. He was born at term with a normal birth weight and had no problems during the prenatal or early postnatal period. His mother and father were not related and there was no history of relevant disease in the family. Physical examination at presentation revealed short stature, nonpalpable testes, and micropenis. Biochemical

investigations confirmed central hypothyroidism accompanied by low prolactin levels. IGF-1, IGFBP-3, and cortisol levels were normal. Treatment was started with L-thyroxine 25 mcg daily. GHSTs, performed after the patient became euthyroid, were compatible with GH deficiency.

MRI scan of the pituitary gland and cranium was normal. Testis USG revealed proximal inguinal located testes with right testis 0.1 mL and left testis 0.2 mL. Renal USG was normal and the patient underwent orchiopexy.

At follow-up at 1.6 years old, growth velocity decreased and GH treatment was started at a dose of 0.03 mg/kg/day. GH induced a remarkable increase in his growth velocity. This patient was suspected to have hypogonadotropic hypogonadism because of low gonadotropin levels, bilateral cryptorchidism, and micropenis at presentation. GnRH stimulation test was performed at the age of 10.5 years and stimulated FSH was 2.27 IU/L, LH was 1.17 IU/L, results which support the diagnosis of hypogonadotropic hypogonadism.

He had a normal sense of smell and no mirror movements of the upper limbs, no abnormal eye movements, no color blindness, and no renal abnormalities or dysmorphic features were noted. At 12.9 years old spontaneous puberty had started and the testis volumes were 4 mL. At the onset of puberty, FSH level was 7.9 IU/L, LH was 1.42 IU/L, and testosterone was 0.112 ng/mL. GH treatment was stopped at 14.1 years old because the patient's height was 173 cm. At final evaluation, the patient was 14.4 years old and the pubertal stage was Tanner 2. Gonadotropin levels, inhibin B (122 pg/mL) and anti-Müllerian hormone levels (7 ng/mL) were in normal ranges. Since GH deficiency continued at retesting, it was decided to continue GH in a dose appropriate for transition.

Patient 4

Patient 4 was referred because of growth retardation, micropenis, cryptorchidism, and hypernatremia at the age of 0.5 years. He was born into a consanguineous family with low birth weight because of oligohydramnios. Physical examination revealed micropenis, nonpalpable testis, scrotal hypoplasia, and short stature. He had severe neuromotor retardation with hypotonia and did not have head control or eye contact. Laboratory evaluation showed hypernatremia with decreased urinary density and increased diuresis which were diagnostic for diabetes insipidus. He had grade 1 pelviectasia on renal USG. Desmopressin treatment was started. Hypophysial axis evaluation revealed prolactin deficiency, hypogonadotropic hypogonadism, and central hypothyroidism. Cortisol response after 1 mcg adrenocorticotropin hormone stimulation test was normal. L-thyroxine treatment was started. On USG, both testes were located inguinally. A human chorionic gonatropin test was performed but testosterone response was inadequate. IGF-1 level was low and GHSTs were compatible with growth hormone deficiency. Cranial and pituitary MRI revealed hypoplasic pituitary and diffuse hypomyelination and cerebral atrophy. GH treatment (0.03 mg/kg/day) was initiated at age 1.9 years old.

Discussion

In this study, we describe four patients with short stature carrying heterozygous variants in the *PROKR2* gene predicted to cause altered function. Patient 1 and Patient 2 had isolated GH deficiency but Patient 3 and Patient 4 had MPHD. Both of the variants p.(Arg85His) and p.(Leu173Arg) have been previously described in patients with IHH, hypothalamic amenorrhea and Kallmann syndrome. However, their role in the etiology of other pituitary hormone deficiencies is unclear.

PROK2 or *PROKR2* variants associated with Kallmann syndrome are usually monoallelic; only a few patients were reported with homozygous or compound heterozygous inheritance (10,13,14). Kallmann syndrome related to heterozygous *PROK2* and *PROKR2* variants is challenging, because knockout mouse models for Kallmann support phenotype in biallelic forms (8). However, functional analyses of monoallelic p.(Leu173Arg) and p.(Arg85His) variants were shown to be deleterious to protein function, supporting a causative role in the clinical outcome (9,10,11,21). Caronia et al. (22) proposed that the monoallelic mutations in *PROKR2* are not sufficient to cause IHH but they could set a lower threshold for functional inhibition of the hypothalamic–pituitary–gonadal axis under adverse hormonal, nutritional, or psychological conditions and thereby lead to hypogonadism. This explanation is compatible with the presence of mutations associated with IHH and hypothalamic amenorrhea in persons who do not have symptoms. For instance, heterozygous *PROKR2* mutations have been reported in patients with IHH, and in many of these patients, the variants were inherited from an asymptomatic parent (10,13,23). An alternative possibility for this variable phenotype of *PROKR2* may be the dominant negative effect of some variants on the normal allele but this mechanism is unlikely to account for the deleterious effect of all missense alterations, as many of them have also been found in healthy individuals (7,11,21,24,25,26). In addition, Monnier et al. (10) reproduced heterozygous *PROKR2* mutations in a recombinant murine *PROKR2* protein and they found that the mutant receptors did not affect cell surface-targeting of the wild-type receptor and did not

properly address the plasma membrane which affects wild-type receptor signaling activity. This finding was evidence against a dominant negative effect of the mutations *in vivo*.

Oligogenic or digenic inheritance has recently been the most plausible explanation for the phenotypes observed in patients with heterozygous mutations in Kallmann syndrome and IHH (12,13,21,24). Few reports of patients carrying mutations in both *PROKR2* and *ANOS1* or in *PROKR2* and *PROK2* supported the digenic inheritance (11,13,23,24,27). Phenotypes resulting from heterozygous *PROKR2* mutations are remarkably variable, ranging from IHH to MPHD with or without abnormalities of the olfactory and optic nerves. Raivio et al. (12) hypothesized that *PROKR2* mutations may underlie both Kallmann syndrome and hypopituitarism because of similar embryonic development and phenotypes of these two entities. They identified patients with MPHD who harbored loss of function variants in the *PROKR2* gene (12). However, the data about the oligogenic inheritance of *PROKR2* in MPHD and isolated GH deficiency is limited.

Additionally, digenic inheritance was shown in some patients as a potential cause of MPHD and pituitary stalk interruption syndrome (12,28,29). If there is incomplete segregation of a heterozygous mutation with the phenotype in a pedigree, digenic inheritance must be considered for the underlying genetic mechanisms (29). To our knowledge and including our cohort, currently 2435 patients with isolated GH deficiency, MPHD, and/or SOD have been investigated for *PROKR2* mutations, and 33 patients (1.4%) harbor 13 different heterozygous *PROKR2* variants (12,28-36). Of these patients, 4 (12%) were reported to have an oligogenic inheritance. Table 3 shows phenotypes of patients with heterozygous *PROKR2* mutations who have MPHD or GH deficiency reported at the time of writing.

In our study, we observed the allele frequency of both variants as 0.017. According to Gnomad database (37,38), the allele frequency of these variants is given as 0.00074 (0.0011-0.00011) for c.254G>A and 0.0023 (0.0063-0.00004) for c.518T>G. In Turkish varioma data (39), which consists mainly of neurological patients, allele frequencies were reported as 0.001 and 0.0036, respectively. In Turkish varioma database, both variants are observed to be 2-10 times higher than the Gnomad frequency but they remain within the frequency ranges of the Gnomad database. In the present study, the allele frequency was found to be dramatically higher than in both databases, although there is a possibility that the frequency will decrease slightly with the increase in the number of patients. The high frequency in our study can be explained by the fact that the phenotype of short stature, which is our patient group, is observed at a higher frequency in the population than in rare diseases.

Study Limitations

The main limitation of this study was the lack of whole exom sequencing, whole genome sequencing, long read sequencing or optical mapping techniques, which are advanced, further step of next generation sequencing and helps to identify underlying additional genes and clarify the etiology. Another limitation was the inability to determine the phenotype-genotype relation and the variability depending on gender because of the small number of patients.

Conclusion

Finally, our data extend previous reports demonstrating that heterozygous *PROKR2* mutations play a role in the etiology of MPHD and isolated GH. Asymptomatic carrier parents and phenotypic variability indicate a yet unknown underlying mechanism of *PROKR2* causing pituitary hormone deficiency. For the mechanisms we have explained in detail above, we concluded that the most likely cause is digenic or oligogenic inheritance in patients with heterozygous *PROKR2* mutations. Although the remaining 24 genes were normal in all patients, we hypothesize our patients carry additional mutations in as-yet-undiscovered Kallmann syndrome or MPHD genes, in the light of all reported data. Besides, the delay in puberty of patients and their relatives may be evidence for *PROKR2* having a role in the constitutional delay of puberty. Further studies are needed to explain in more detail the role of PROKR2 signaling in the reproductive system and pituitary development.

Acknowledgements

The authors wish to express their gratitude to the parents and the patients who participated in this study.

Ethics

Ethics Committee Approval: The study protocol was approved by the İstanbul University, İstanbul Faculty of Medicine Local Clinical Research Ethics Committee (date: 11.08.2017, approval number: 13).

Informed Consent: Written informed consent was obtained from all patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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Financial Disclosure: This research project was supported by the Scientific Research Projects under numbers TYL-2017-26405, and TDK-2022-38742.

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