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PROKR2 Mutations in Patients with Growth Hormone Deficiency and Multiple Pituitary Hormone Deficiency

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BACKGROUND

Prokineticin receptors (*PROKR1* and *PROKR2*) belong to the family of G protein-coupled receptors. Bi- or mono allelic mutations in *PROKR2* gene (20p12.3, NM_144773.2) have been identified in Kallmann syndrome or idiopathic hypogonadotropic hypogonadism (IHH) which is characterized by hypogonadotropic hypogonadism (HH) with or without anosmia/hyposmia (1). Recently, *PROKR2* mutations were reported in patients with multiple pituitary hormone (MPHD) and growth hormone deficiency (GHD), suggesting a potential role for the PROK2 pathway in pituitary development, in addition to its role in GnRH neuron development (2). We present here clinical and molecular findings of one patient with MPHD and two patients with GHD.

PATIENTS AND METHODS

Patien 1 and **Patient 2** were presented with short stature (height SDS <- 2) and **Patient 3** was diagnosed with central hypothyroidism at age of 5 months and started on L-T4 replacement therapy and referred for further endocrinological evaluation.

Clinical findings of the patients are summarized in **Table1.** All patients were born at term. There were no dysmorphic findings, mental retardation and anosmia or hyposmia in the patients. All patients had normal vision and hearing. In the family history, there were short stature and delayed puberty.

Six months after presentation, **Patient 1** and **Patient 2** showed a low height velocity and growth hormone (GH) stimulation tests were performed. GHD was diagnosed and GH replacement therapy was started. **Patient 1** and **Patient 2** have completed pubertal **development, menarche age of Patient 1** was 13.5 years and **Patient 2** was 15 years. **Patient 3** is still prepubertal. This patient was suspected to have hypogonadotropic hypogonadism without anosmia because of low gonadotropin levels, bilateral cryptorchidism and micropenis at presentation. Prolactin (PRL) level was also low (1.9 ng/ml). Dihydrotestosterone cream was applied for micropenis. GHD was diagnosed at presentation, but GH treatment was started at age of 2.2 years and orchiopexy was done at age of 2.7 years. GnRH stimulation test was performed at age of 10.5 years. LH and FSH responses were very low, these results have been supported to hypogonadotropic hypogonadism.

Written informed consent was obtained from the patients and their parents for genetic analyses.

Chromosomal abnormalities using microarray and cytogenetic techniques were excluded before the admission of molecular genetic analysis. Screening of targeted regions for in-house designed short stature panel with 25 genes (***BMP4***, ***FGF8***, ***FGFR, GH1***, ***GHR, GHRH***, ***GHSR***, ***HESX1***, ***HHIP, IGF1, IGF1R, IGFALS, IGFBP3***, ***IGSF1, LHX3, LHX4***, ***OTX2***, ***POU1F1***, ***PROKR2, PROP1, SHH, SHOX, SOX3, STAT5B, WDR11)*** and tested using Ion Torrent PGM™ system for next-generation sequencing (ThermoFisher Scientific, Waltham, MA, USA). Genetic analyses revealed two different heterozygous clinical variants previously reported with Kallmann syndrome in each patient in *PROKR2* gene. **Patient 1** and **Patient 2** had **heterozyous p.Arg85His** mutation. **Patient 3** had **heterozyous p.Leu173Arg** mutation. Family genetic analyses revelead that this mutation was transmitted from her father in **the Patient 1,** his mother in the **Patient 2.** The mother of **Patient 3** was carrier for p.Leu173Arg mutation.

DISCUSSION

It is reported that the phenotypes resulting from heterozygous *PROKR2* mutations are remarkably variable, ranging from isolated GnRH deficiency to MPHD with or without abnormalities of the olfactory and optic nerves. Oligogenic or digenic inheritance is recently to be the most plausible explanations for the phenotypes observed in patients with heterozygous mutations (1,2). The *PROKR2* p.Arg85His and p.Leu173Arg mutations described previously have been associated with IHH. Functional analyses were performed for two mutations and shown to be deleterious to protein function, supporting a causative role in the phenotype. It was reported that these mutations were inherited heterozygously from asymptomatic parents to several patients with IHH or hypothalamic amenorrhea. A male patient with IHH who had *PROKR2* mutation underwent spontaneous reversal of his GnRH deficiency and suggests that gene-environment interaction may modify a phenotype later in life (3,4,).

Patent 1 and **Patient 2** had a slightly delayed menarche. Interestingly, **Patient 1**'s mother was not carrier the mutation, but she had delayed menarche and mildly short stature. This condition may be related to other causes. Patient 2's mother who was carrier for the mutation had short stature and delayed puberty. Patient 3's mother who was carrier for the mutation did not have short stature, but her manarche age was slightly delayed. There were phenotypic differences for two *PROKR2* variants carriers in the intrafamily and interfamily members. It may be possible because of *PROKR2* gene expressivity difference. It may contribute oligogenic or digenetic inheritance.

CONCLUSION

- Heterozygous *PROKR2* mutations should be kept in mind as a very rare cause of GHD and MPHD. Futher studies are needed to explain in more detail the role of PROKR2 signalling in reproductive system and pituitary development.
- Phenotypic variability was seen in family members with the same mutation, including asymptomatic or milder phenotype.

Table 1. Some clinical and laboratory findings of the patients

	Patient 1	Patient 2	Patient 3
Clinical findings at presentation			
Age (yrs)	12	11	0.5
Gender	Female	Female	Male
Consanguinity	3rd degree	1st degree	No
Birth weight g/SDS	2500 / -2.0	3600 / 0.7	3230 / -0.6
Height cm/SDS	135 /-2.7	128.4/-2.5	59.2/-3.3
Weight kg/SDS	30.9 /-2.0	31.4/-1.0	8.25/0.05
BMI SDS	-0.8		
Head circumference cm/SDS	52.8/-0.8	51.6/-1.2	43.2/-0.7
Sitting height/Height (cm/cm)	0.54	0.53	0.69
Pubertal stage (Tanner stage)	A1Ph1B2/2	A1Ph1B2/2	A1Ph1T0.5/0.5 ml
Bone age (yrs)	$8^{10/12}$-10	$7^{10/12}$-$8^{10/12}$	1 (at age of 1.6 yrs)
Mother height SDS	150.6 /-1.9	147.4/ -2.4	157.5 / -0.9
Father height SDS	158.8 /-2.4	167.2 /-1.3	168.8 /-1.1
Target height cm/SDS	148.2/-2.3	150.8/-1.9	169.7/-0.9
Associated findings	-	-	Cryptorchidism and micropenis
Familiy history			
Mother's menarche of age (yrs)	16	15	14
Short stature (by history)	Parents 2 Mother's sisters	Mother's sister and some relatives	Grandmother (Mother's mother)
Delayed puberty	Mother Brother	Mother	
At recent evaluation			
Age (year)	14.6	18.4	10.4
Height cm/SDS	146.4/-2.5	153.2/-1.7	154.1 / 2.3
Weight kg/SDS	55.4/0.1	57.3/-0.1	60.9 / 2.5
Sitting height / Height	0.54	0.52	0.54
BMI SDS	1.63	1.1	2.1
Pubertal stage (Tanner stage)	A3Ph3B4/4	A3Ph5B5/5	A2Ph3T2/2ml
Bone age (yrs)	15	16	13
Hormonal deficiencies	GH	GH	GH-TSH-PRL-Gn
Menarche age (yrs)	13.5	15.6	-
Magnetic resonance imaging (Cranial and pituitary)	Normal	Anterior pituitary gland hypoplasia (height :4.5 mm)	Normal
Genetic analyses			
PROKR2 **NM_144773.2NP_658986.1**	Heterozygous c.254G>A; p.Arg85His CM06540 rs74315418	Heterozygous c.254G>A; p.Arg85His CM06540 rs74315418	Heterozygous c.518T>G; p.Leu173Arg CM065404 rs74315416

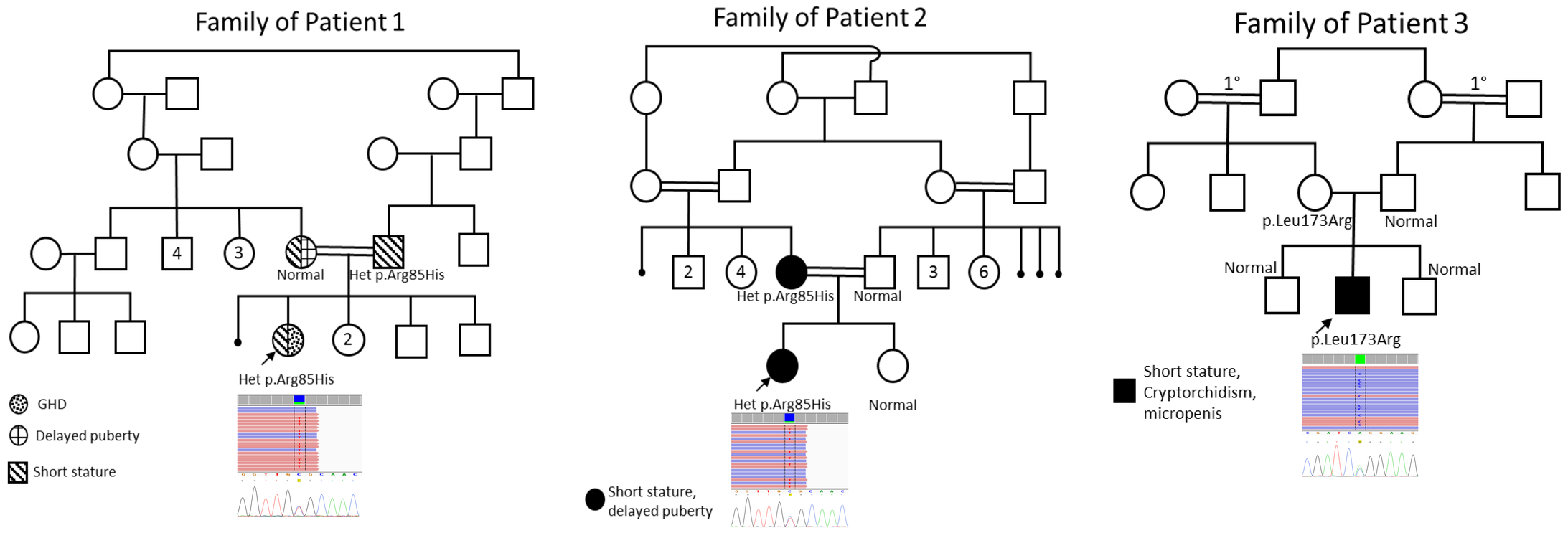

Figure 1. Family pedigrees of the patients with *PROKR2* allelic variants

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